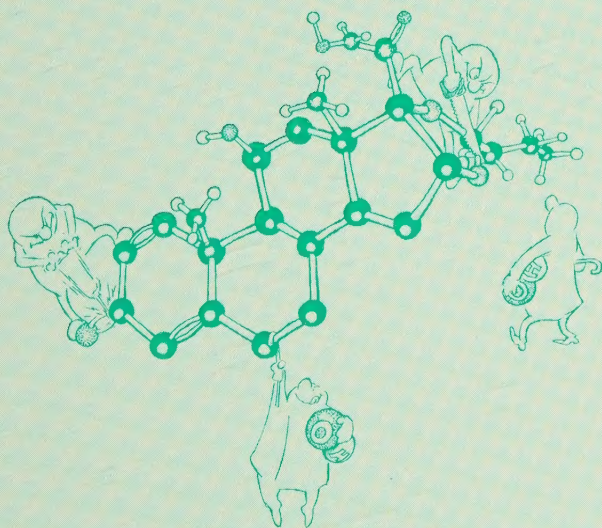


Studies on the Metabolic Fate and Human Pharmacokinetics of Budesonide

Staffan Edsbäcker



Diss. Lund

1986



Digitized by the Internet Archive
in 2026

From the Department of Clinical Pharmacology,
University of Lund, Lund, Sweden

STUDIES ON THE METABOLIC FATE AND
HUMAN PHARMACOKINETICS OF BUDESONIDE

by

Staffan Edsbäcker

Civ.ing., Hb.

AKADEMISK AVHANDLING

som för avläggande av doktorsexamen i medicinsk vetenskap
vid Lunds Universitet kommer att offentligen försvaras
i föreläsningssal 3, Lunds lasarett
torsdagen den 29 maj 1986 kl 09.00

Organization LUND UNIVERSITY Dep. of Clinical Pharmacology University of Lund S-221 85 Lund Sweden		Document name DOCTORAL DISSERTATION	
		Date of issue 1986-04-09	
		CODEN: LUDEDW/(MECF-1010)/1-62/(1986)	
Author(s) Staffan Edsbäcker		Sponsoring organization AB Draco, Box 34, S-221 00 Lund, Sweden	
Title and subtitle Studies on the metabolism and human pharmacokinetics of budesonide			
Abstract The metabolic fate and some pharmacokinetic properties of the topical glucocorticoid budesonide have been studied. The drug (a 1:1 mixture of epimers 22R and 22S) was extensively biotransformed in human, mouse and rat liver 9000g supernatant fraction. Of the two animal species studied, the liver metabolism in the mouse was most similar to that of man. The main metabolic pathway in human and mouse livers, the 16α,17α-acetal splitting, is unique within this group of drugs. The biotransformation did proceed via an intermediary ester, giving 16α-hydroxyprednisolone and butyric acid. It was substrate selective for epimer 22R. Other metabolic pathways did include 6β-hydroxylation, Δ^6-dehydrogenation, A-ring reduction and acetal side chain hydroxylation. After intravenous administration of budesonide to healthy subjects, the apparent volume of distribution was 2.8 L/kg, plasma clearance 0.9 L/min and plasma half-life 2.5 h. The major metabolite in plasma and urine was 16α-hydroxyprednisolone. The systemic availability after nasal instillation was 102%. The budesonide epimers had different pharmacokinetic and metabolic properties in man. The apparent volume of distribution and plasma clearance of epimer 22S was significantly less than the corresponding values of epimer 22R. Different serum binding and liver biotransformation rates were contributing factors to this disparity between the epimers.			
Key words Budesonide - glucocorticoid - liver biotransformation - metabolism - pharmacokinetics - nasal administration - serum binding			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN 91-7900-070-3	
Recipient's notes		Number of pages	Price
		Security classification	

Distribution by (name and address) Staffan Edsbäcker, Pharmacokinetics Laboratory, Research & Development Dep., AB Draco, Box 34, S-221 00 Lund, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Staffan Edsbäcker

Date 9/4 1986

From the Department of Clinical Pharmacology,
University of Lund, Sweden

Studies on the Metabolic Fate and Human Pharmacokinetics of Budesonide

Staffan Edsbäcker



Lund 1986

To Eva, Lina and Jatzy

This thesis is based mainly on the following papers, which will be referred to in the text by their Roman numerals (I-VI);

- I. Edsbäcker S, Jönsson, Lindberg C, Ryrfeldt Å & Thalén A (1983). Metabolic pathways of the topical glucocorticoid budesonide in man. *Drug Metab Disp* 11, 590-596.
- II. Edsbäcker S, Andersson P, Lindberg C, Paulson J, Ryrfeldt Å & Thalén A. Liver metabolism of budesonide in the rat, mouse and man; comparative aspects. Submitted for publication.
- III. Edsbäcker S, Andersson P, Lindberg C, Ryrfeldt Å & Thalén A. Metabolic acetal splitting of budesonide - a novel inactivation pathway for topical glucocorticoids. Submitted for publication.
- IV. Edsbäcker S, Andersson KE & Ryrfeldt Å (1985). Nasal bioavailability and systemic effects of the glucocorticoid budesonide in man. *Eur J Clin Pharmacol* 29, 477-481.
- V. Ryrfeldt Å, Edsbäcker S & Pauwels R (1984). Kinetics of the epimeric glucocorticoid budesonide. *Clin Pharmacol Ther* 35, 525-530.
- VI. Edsbäcker S & Geelmuyden K. Serum protein binding of the budesonide epimers in man. In manuscript.

CONTENTS

INTRODUCTION

Glucocorticoid hormone action.....	1
Place in therapy of glucocorticoids.....	2
Budesonide - chemical, pharmacological and therapeutic properties.....	4
Metabolism and pharmacokinetics of glucocorti- coids - introductory remarks.....	5

AIMS

MATERIAL AND METHODS

Radiolabelled compounds.....	8
In vitro experiments.....	8
Liver incubations.....	8
Metabolite identification.....	8
Estimation of oral availability from in vitro data.....	9
Cimetidine interaction.....	9
Serum binding.....	9
Human studies.....	9
Subjects.....	9
Administration and sampling procedures.....	9
White blood cell counts and plasma cortisol....	10
HPLC.....	10
Quantitation of budesonide and metabolites.....	10
Pharmacokinetic calculations.....	11

RESULTS

In vitro metabolism of budesonide in liver 9000g supernatant fraction	13
Metabolic acetal splitting of budesonide	16
Systemic elimination of budesonide in man; metabolic aspects	18
Nasal bioavailability of budesonide in man	20
Pharmacokinetics and serum binding of the budesonide epimers	21
Systemic effects after bolus doses of budesonide ...	22

GENERAL DISCUSSION

Metabolism of glucocorticoids.....	23
Metabolism of cortisol.....	23
Metabolism of synthetic glucocorticoids, including budesonide.....	24
Some metabolic structure-activity concide- rations.....	28
Glucocorticoid pharmacokinetics in man.....	28
Summary of basic kinetics.....	28
- deposition and absorption.....	28
- disposition.....	29
- excretion.....	31
Disposition of budesonide epimers 22R and 22S..	32
- in vitro aspects.....	32
- in vivo aspects.....	32
Factors regulating the disposition and metabolic inactivation of glucocorticoids.....	34
Species and sex differences.....	34
Drug interactions.....	35
Diseased state.....	36
Other factors.....	37
Topical to systemic effect ratio - pharmacokinetic concepts.....	38

SUMMARY AND CONCLUSION.....	42
-----------------------------	----

ACKNOWLEDGEMENTS.....	44
-----------------------	----

REFERENCES.....	46
-----------------	----

APPENDIX (papers I-VI)

ABBREVIATIONS

AMP	Adenosine monophosphate
β	Terminal plasma rate constant
BDP	Beclomethasone-17 α ,21-dipropionate
C	Plasma concentration
CL	Clearence
CNS	Central nervous system
dep.	Dependent
E	Extraction ratio
epil.	Epileptic
F	Female(s)
F _O	Oral availability
fu	Serum free fraction
fu _r	Tissue free fraction
GC	Glucocorticoid
GCR	Glucocorticoid receptor
GI	Gastro-intestinal
h	Hour(s)
HPA	Hypothalamus pituitary adrenal
HPLC	High pressure liquid chromatography
i.t.	Intratracheal
i.v.	Intravenous
K _L	In vitro biotransformation rate constant
K _M	Michaelis constant
K _U	Terminal urinary rate constant
LC	Liquid chromatography
M	Male(s) or Molar
maint.	Maintenance
min	Minute(s)
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
NADPH	Reduced nicotineamide dinucleotide phosphate
NMR	Nuclear magnetic resonance
pept.	Peptic
p.o.	Per oral
psych.	Psyciatric
S.D.	Standard deviation
SEM	Standard error of the mean

$t_{1/2}$	Half life
$T_{\max}$	Time for maximal plasma concentration
V_{β}	Volume of distribution calculated by use of β
$V_{\max}$	Maximal biotransformation velocity
V_{ss}	Volume of distribution calculated by moment analysis
WBC	White blood cell(s)

INTRODUCTION

Glucocorticoid hormone action

The term glucocorticoid (GC) relates to the glucose-regulating properties of adrenal cortical steroid hormones. These properties are manifested as a peripheral glucose-sparing effect, increased storage of glucose as liver glycogen and protection of glucose dependent central functions. The effects of GC hormones on body functions at physiological and pharmacological concentrations include an anabolic effect on the liver with increased gluconeogenesis and enzyme synthesis, a catabolic effect on many other tissues, a redistribution of body fat and increased lipolysis, a negative calcium-balance, certain CNS and cardiovascular actions, effects on fluid and electrolyte balance, effects on insuline and adrenergic hormone action, effects on blood erythrocyte and leucocyte content and a general lympholytic effect. (cf Haynes & Murad 1980 or Baxter & Rousseau 1979). The influence of glucocorticoids on protein, lipid and nucleic acid metabolism play important roles in regulation of homeostasis, metabolism, growth and development, and responses to stress (cf Ganong 1981a).

Cortisol, the major endogenous GC in man, is synthesized in the fasciculata and reticularis zones of the adrenal cortex from cholesterol via pregnenolone and 17α -hydroxyprogesterone. The biosynthesis of cortisol is regulated by adrenocorticotropical hormone (ACTH), which is secreted in circadian rhythms by the adenohypophysis. ACTH stimulates the formation of free cholesterol in the adrenal gland as well as stimulates the cyclic AMP-mediated increase in oxidative cleavage of the cholesterol side chain - the rate-limiting step in the formation of cortisol (the biosynthesis and regulation of GC hormones have been reviewed by Haynes & Murad 1980).

The currently adopted theory for the mechanism of action of GCS and other steroid hormones, is that after diffusion of the steroid over the cell membrane, it binds to a receptor protein

in the cytoplasm. The steroid-receptor complex is translocated into the cell nucleus where it interacts with the chromatin (Higgins et al. 1979). The sub-cellular localization of steroid receptors have however recently been debated (Clark 1984). Specific mRNA is synthesized and after translation, the protein formed will lead to the specific biological effects exerted by the steroid in question (Higgins et al. 1979).

Place in therapy of glucocorticoids

In 1949 it was discovered that i.v. cortisone, a compound first isolated by Reichstein et al. (cf Reichstein & Shoppee 1943), had an anti-rheumatic effect (Hench et al. 1949). In the following year, the beneficial effect of i.v. cortisone on bronchial asthma and hay fever could be demonstrated (Carrier et al. 1950). These discoveries were followed by an explosive growth in steroid research and it soon became apparent that cortisol, the pharmacologically active and major cortisone metabolite, could be used in the treatment of a variety of clinical conditions, including asthma, rhinitis and skin diseases.

Currently, GCs are among the most important drugs available (cf Baxter & Rousseau 1979 or Haynes & Murad 1980). The anti-inflammatory and immunosuppressive effects in pharmacological doses are the most important therapeutic properties (Fauci 1976, Melby 1977). Treatment is however generally palliative, and the underlying cause of the disease usually remains. Hence, by some, GCs have been referred to as "agents which permit the patient to walk to the autopsy room" (Haynes & Murad 1980). Furthermore, prolonged treatment with high doses of systemic GCs may be associated with side effects, including muscle atrophy, osteoporosis, changed distribution of body fat, unmasking of latent diabetes mellitus, hypertonia, adverse psychiatric reactions and reactivation of latent infections (cf Swartz & Dluhy 1978 or Siegel 1985). Such adverse effects, referred to as Cushingoid symptoms (Baxter & Rousseau 1979), have restricted a more general use of these pharmacologically powerful agents.

By synthetically modifying the basic structure of cortisol, the therapeutic effects of GCs have been increased, and the unwanted side effects diminished. In this way, the mineralcorticoid activity, i.e. the effects on fluid and electrolyte balance, has been almost abolished. Following the introduction of synthetic GCs with high topical potency and rapid systemic inactivation for local use in e.g. asthma and rhinitis, the fear of severe side effects have gradually abated (cf Tse & Bernstein 1984, Mygind 1982).

Asthma has been defined as "reversible obstructive airways disease of unknown etiology until proved otherwise" (Farr 1985). This definition reflects the unclear state of knowledge of the disease. Pathological changes in asthma include bronchospasm, mucosal edema, airway hyperreactivity, mucus secretion, desquamation and basement membrane thickening (Kaliner 1985). GCs are believed to have effects on most of these changes, primarily by anti-inflammatory and anti-hyperreactivity actions (Hanson & Morley 1985). This is expressed as a suppressed accumulation of neutrophils and other pro-inflammatory cells and agents in areas of inflammation, constriction of microvasculature, inhibition of the synthesis of arachidonate metabolites and platelet activating factor by inhibition of phospholipase A₂ activity, inhibition of histamine release and a permissive effect on adrenergic β_2 -receptors (cf Morris 1985 and Hanson & Morley 1985).

Topical GCs in the treatment of non-infectious rhinitis has meant a major therapeutic advance (Mygind 1982). The pharmacological effects of these agents in rhinitis are similar to those in asthma, i.e. decreased vasodilatation and decreased edema, reduced amount in the nose of pro-inflammatory cells and inhibition of immediate and late allergen-induced reactions (Pipkorn 1982, Mygind 1982).

GCs are used topically in the treatment of allergic and/or inflammatory skin diseases. Anti-inflammatory action, including vasoconstriction, is together with an anti-proliferative action

on epidermal cells, the major therapeutic effects of these agents in skin diseases (Lorenzetti 1981). Local effectiveness of GCs in skin disorders depends not only on the intrinsic potency of the agent but also on the ability to penetrate the epidermis. Many of the potent dermal GC formulations have however been connected with side effects, such as dermal atrophy and HPA-axis suppression (Lorenzetti 1981, Fritz & Weston 1983). The effects of GCs on fibrous connective tissue have been studied by Särnstrand (1985).

Budesonide - chemical, pharmacological and therapeutic properties

Budesonide (FIG.1) is a lipophilic glucocorticoid $16\alpha,17\alpha$ -acetal, consisting of a 1:1 mixture of two epimers with 22R and 22S configuration (Thalén & Brattsand 1979).

In animal models, both epimers have a favourable ratio between topical and systemic GC effects (Brattsand et al. 1982a). The high topical potency is correlated with a high GC receptor affinity, exceeding that of cortisol about 200 times (Dahlberg et al. 1984). The drug has a protective effect on lung anaphylaxis in rats (Dahlbäck et al. 1986), and in guinea-pigs

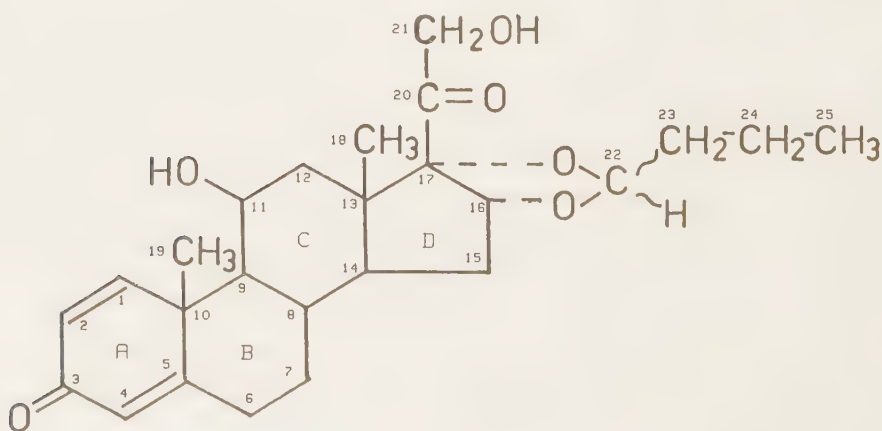


FIG.1. STRUCTURAL FORMULA OF BUDESONIDE.

(Andersson et al. 1982a, 1982b, Forsberg et al. 1982), and is furthermore effective in preventing the late allergic response to antigen in guinea-pigs (Brattsand et al. 1985a) and in sheep (Lanes et al. 1985). An effect on both the immediate and late allergic reaction has been demonstrated also in man (Dahl & Johansson 1982). The high topical potency (Gruvstad & Bengtsson 1980) and the favourable ratio between topical and systemic effects have been confirmed in human studies (Johansson et al. 1982).

Budesonide is presently used in the local treatment of asthma (Johansson 1983), rhinitis (Pipkorn et al. 1980) and skin disorders (Agrup et al. 1981). Treatment has been connected with a high therapeutic efficacy, but with no or marginal side effects also after prolonged treatment (cf Clissold & Heel 1984). Human pharmacokinetic studies have revealed a rapid absorption from the lung, a high body clearance and a large first pass elimination after oral administration. Plasma binding is about 90% and plasma/erythrocyte ratio close to unity (Ryrfeldt et al. 1982).

Metabolism and pharmacokinetics of glucocorticoids - introductory remarks

The human liver is supplied with enzymatic capacity to eliminate endogenous GCs almost completely (Dorfman & Ungar 1965). The same or similar routes of elimination have been demonstrated for many synthetic GCs (Fortherby & James 1972).

Cytochrome P-450 is a vital system of enzymes in drug and steroid metabolism (Hall 1980). It is present in various organs but primarily in the liver. Cytochrome P-450 has the ability to incorporate into molecules one oxygen atom from atmospheric oxygen, rendering the molecule more polar and hence more liable to excretion. This biotransformation may also precede other metabolic events such as conjugation reactions (the properties of cytochrome P-450 has been reviewed by Mannering 1981).

The hitherto most utilized GC in the local treatment of respiratory diseases, beclomethasone-17 α ,21 α -dipropionate (BDP) is biotransformed by hydrolysis in several tissues to a pharmacologically active metabolite, BMP (Andersson & Ryrfeldt 1984). There are several other examples in the literature on the formation of pharmacologically active or toxic drug metabolites (cf. Drayer 1982 or Garattini 1983), and generally metabolic documentation of new drug entities is required by the authorities (Case 1981).

Metabolism of drugs and endogenous compounds can be studied in vivo in intact animals and in man. By identifying and quantifying plasma and excretory products of the compound, the routes and extent of metabolism can be estimated. Metabolic studies performed in vitro, in isolated perfused organs, isolated cells or cell cultures and tissue homogenates, allows more comprehensive conclusions to be drawn concerning mechanistic aspects, sites of metabolism and the influence of e.g. genetic and environmental factors.

Topical drug treatment is based on the pharmacokinetic concept that local administration requires less active drug for therapeutic efficacy, which in turn diminishes the risk for unwanted effects elsewhere in the body. This concept has served as the basis for the fairly recent and ongoing research on topically potent GCs having high rates of systemic inactivation. The correlation between various GC responses and plasma levels of active compound(s) are however complex and far from being understood. Obviously the intricate biochemical events preceeding GC action, and the abundance of sites from which GC activity might emanate, contributes to this complexity. Still, pharmacokinetic considerations is of major importance for the understanding of the pharmacodynamics and clinical efficacy of topical GCs.

Not until recently has sensitive analytical assays been developed for budesonide. Pharmacokinetic studies, including those presented here, have therefore been restricted to the use of tritium-labelled compound.

The main purpose of the present investigation was to study the fate of budesonide in vitro in liver homogenates from different species including man and in vivo in healthy subjects.

Specifically, the objectives were to

- study the liver metabolism of budesonide, focusing on the metabolic consequences of the modified $16\alpha,17\alpha$ -acetal side chain in budesonide,
- study differences in liver metabolism between rat, mouse and man,
- elucidate the disposition, the major metabolic pathways and the routes of excretion after intravenous dosing in man,
- consider some aspects of budesonide absorption and metabolism in a target organ - the nose, and
- exploit some pharmacokinetic implications of budesonide being a mixture of two epimers.

MATERIAL AND METHODS

Only a summary of pertinent data will be given. The reader is referred to each individual paper for a full description of the material and methodology used.

Radiolabelled compounds

[³H]Budesonide, tritium-labelled in 1,2- or 22-25-position, was used in the radioactive experiments. [³H]Budesonide epimers were obtained by HPLC-separation (see below). Prior to each study, the radiochemical purity was checked by HPLC, and found to exceed 93%.

In vitro experiments

Liver incubations. Specimens of 7 human livers were obtained from Huddinge Hospital, Sweden by the courtesy of Dr. C. von Bahr. The livers were taken shortly after circulatory arrest, immediately frozen at -196°C and then stored at -80°C before use. Livers from Sprague Dawley CR rats and NMRI mice were perfused in situ with ice-cold phosphate buffer pH 7.4 and then removed. Livers were minced, weighed and homogenized in phosphate buffer pH 7.4. The homogenates were centrifuged at 9000g for 20 min. Cytosolic fractions were prepared by centrifugation of the 9000g supernatant fraction for 1 h at 100000g. Incubations were performed by gentle shaking at 37°C under carbogen or oxygen atmosphere.

Metabolite identification. Metabolites, formed after in vitro incubation of budesonide with liver 9000g supernatant fractions from rat, mouse and man, were extracted with ethylacetate or disposable Sep-Pak C₁₈ cartridges, and submitted to HPLC separation (see below). Chemical ionization (methane) mass spectra of isolated metabolites and synthetic reference compounds were obtained using either a direct inlet probe or a moving belt

LC-MS interface. Stable isotopes ($(^2\text{H}_8)$ -budesonide and ^{18}O -gas) were used to facilitate interpretation of mass spectral data. ^1H -NMR was used for identification of some of the isolated metabolites.

Estimation of oral availability from in vitro data. K_M and V_{MAX} of (22R)- and (22S)-budesonide in human liver 9000g supernatant fraction (protein concentration 1 mg/mL) were estimated after incubation of [^3H]budesonide at 2-20 μM and HPLC-separation of the epimers. Experiments were performed with two human livers. Complete recovery of metabolic capacity was assumed. In addition, binding to liver proteins was assumed not to influence metabolic activity.

Cimetidine interaction. [^3H]Budesonide was incubated with human liver 9000g supernatant fraction as described above, in the presence and absence of cimetidine. Liver protein concentration was 1 mg/mL. Biotransformation rates were calculated at different initial concentrations of budesonide (2-20 μM) and cimetidine (0.5-2 mM) after 0, 5 and 10 min of incubation.

Serum binding. Protein binding of the [^3H]budesonide epimers were investigated by equilibrium dialysis, using serum from 12 healthy subjects. Experiments were also performed using ultrafiltration and ultracentrifugation.

Human studies

Subjects. A total of 10 male subjects, 22-46 years old and weighing 63-95 kg participated. They were all healthy as shown by routine medical examination including hematological and biochemical tests. The subjects did not take any drugs or alcohol for at least four days preceeding budesonide intake. The study protocols were approved by the local Ethics committee before initiation.

Administration and sampling procedures. [^3H]Budesonide (50 μCi), 100 and 500 μg , was given by intravenous infusion over 5 and 10

min, respectively. Nasal administration was performed by instillation into each nostril of 50 μ L of a Tween 20 solution of [3 H]budesonide (1 mg/mL, 0.5 mCi/mg). Placebo was given on the day preceeding [3 H]budesonide administration. Blood was collected in heparinized tubes at defined time intervals after placebo and drug administration. Plasma was prepared and rapidly frozen. After drug administration, urine was quantitatively collected in fractions during 24 or 96 hours. In one study, faeces was collected for 96 hours. Samples were stored frozen until analyzed.

White blood cell counts and plasma cortisol. Total and differential blood cell counts and plasma cortisol levels were estimated in plasma samples obtained after placebo and drug administrations. Plasma cortisol levels were estimated by radioimmunoassay.

HPLC

Liquid chromatographic separation was performed on a 5 x 200 mm column packed with Nucleosil C₁₈ 5 μ m particles, using isocratic or gradient elution with ethanol/water or ethanol/buffer mixtures. One min fractions were collected for radioactivity measurements. In some experiments, an on-line radioactivity detector was used.

Quantitation of budesonide and metabolites

Total radioactivity was assessed in all serum, plasma, urine, faecal and liver samples. Internal standard (unlabelled budesonide) was added to plasma and urine samples, and extraction was performed with dichloromethane or Sep-Pak C₁₈. Extraction recoveries of budesonide were >94%. Standard curves were made using blank plasma and urine, to which known amounts [3 H]budesonide were added. Quantification of plasma metabolites was performed in a similar way as quantification of plasma budesonide, except that the metabolite recovery, relative to budeso-

nide, was assessed and compensated for in each sample. The recovery of 16α -hydroxyprednisolone and 6β -hydroxybudesonide as compared to budesonide was $88.3 \pm 2.5\%$ ($n=28$) and $102.3 \pm 2.6\%$ ($n=30$), respectively. Extraction recovery of radioactivity in fractionated 0-24 h urinary samples was $102.3 \pm 3.2\%$ ($n=24$).

Incubated liver samples were extracted with Sep-Pak C_{18} after addition of internal standard. Mean extraction recoveries of total radioactivity were 92, 83 and 89% for incubations with rat, mouse and human liver preparations. No decrease in extraction recovery was observed after prolonged incubation time, indicating similar recovery of parent compound and formed metabolites. Metabolite concentrations were calculated relative to that of the parent compound at 0 min incubation time.

Radioactivity was measured on line using a Packard Trace 7140 detector, or off line by the liquid scintillation technique. The liquid scintillation detector was equipped with external standard for determination of counting efficiency.

Pharmacokinetic calculations

Area under the plasma concentration time (C,t) curve (AUC) was calculated over the first 8 hours using the trapezoidal rule. The extrapolated AUC ($AUC_{8-\infty}$) was calculated as C_8/β , where β is the terminal slope of the log-linear C,t-curve. Plasma clearance (CL) was calculated as dose divided by $AUC_{0-\infty}$. Volume of distribution was calculated as $V_\beta = CL/\beta$. T_{max} is the time for maximal plasma concentration, $t_{1/2}$ is $\ln 2/\beta$ and $t_{1/2,urine}$ is $\ln 2/K_u$ where K_u is the slope of the log-linear urine excretion rate time curve. Fraction budesonide remaining in the body and in plasma after i.v. administration was calculated as $C \cdot V_\beta / \text{dose}$ and $C \cdot V_p / \text{dose}$, respectively, where V_p is the plasma volume. Linear conditions and instantaneous distribution were assumed.

Fraction budesonide in serum, extracellular fluids and intracellular sites was calculated using the equation of Øje and Tozer (1979): $V = 7 + 8fu + 27fu/fu_r$, where V is the volume of

distribution, f_u the free fraction in serum and f_{u_r} the free fraction in tissues. The following assumptions were made: instantaneous distribution of budesonide and log-linear elimination (i.e. $V = V_\beta$); uniform distribution throughout the total body water space and; serum binding restricted to albumin.

In vitro biotransformation rate, expressed as $t_{1/2}$, was calculated as $\ln 2/K_L$, where K_L is the slope of the log-linear decline in concentration of the parent compound during incubation. Intrinsic clearance and extraction ratio (E) were calculated according to Rane et al. (1977), using the liver weight and liver blood flow suggested by Boxenbaum (1980). Oral availability (F_o) was calculated as $F = 1-E$.

RESULTS

In vitro metabolism of budesonide in liver 9000g supernatant fraction

The metabolism of [^3H]budesonide was studied in vitro in liver 9000g supernatant fraction from rat, mouse and man. Structure elucidation was based on MS-, NMR-, UV- and HPLC retention data. Suggested metabolic pathways of budesonide are given in FIG.2. The two budesonide C-22 epimers produced different metabolites. This was explained by substrate selective oxidation of the non-symmetric 16 α ,17 α -acetal substituent. Epimer 22R gave 16 α -hydroxyprednisolone (metabolite VI) while epimer 22S produced a metabolite tentatively identified as 23-hydroxybudesonide (metabolite VII). Otherwise budesonide followed the general metabolic pathways reported for synthetic GCs. Thus, oxidative metabolism predominated, 6 β -hydroxybudesonide (metabolite V) and Δ^6 -budesonide (metabolite IV) being identified in all investigated species. Metabolite III is oxidized in the steroid nucleus as well as reduced in the A-ring. 4,5 β -Dihydrobudesonide (metabolite II) and 3,4,5 β -tetrahydrobudesonide (metabolite I) were formed primarily in rat liver. Of the two animal species studied, the biotransformation of budesonide in the mouse was most similar to that of man.

When the metabolic pattern, obtained after incubation of [^3H]budesonide with human liver preparations, was compared with the metabolite pattern in plasma obtained after i.v. administration of [^3H]budesonide to man, no qualitative difference could be found (FIG.3). This indicates that the in vitro model is useful to predict results in vivo.

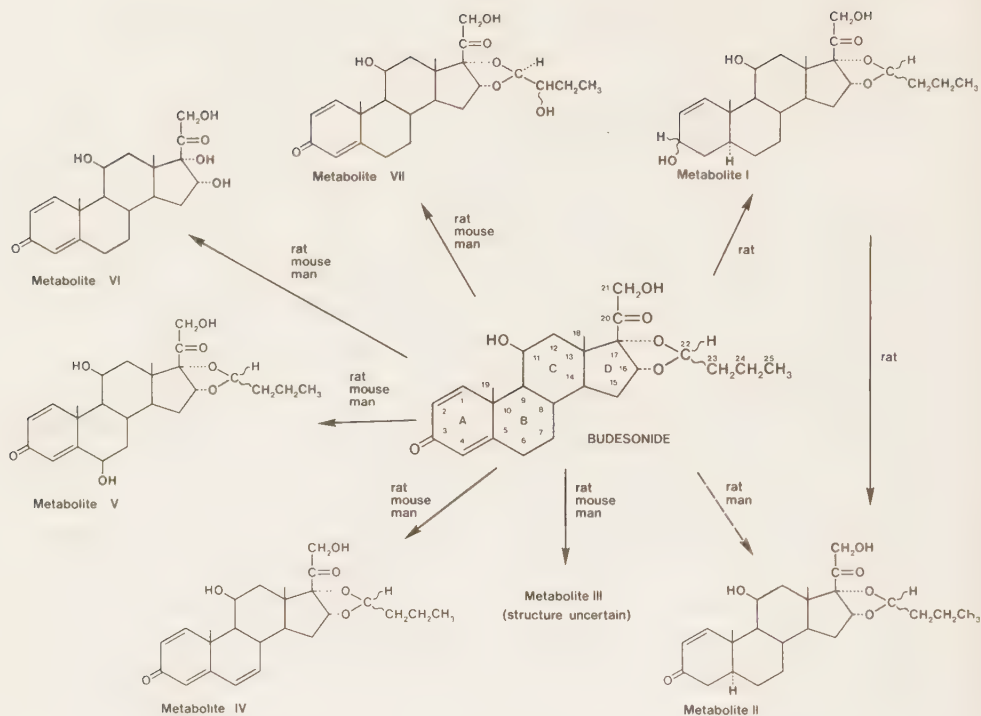


FIG.2. SUGGESTED METABOLIC PATHWAYS OF BUDESONIDE IN LIVER 9000g SUPERNATANT FRACTION FROM RAT, MOUSE AND MAN.

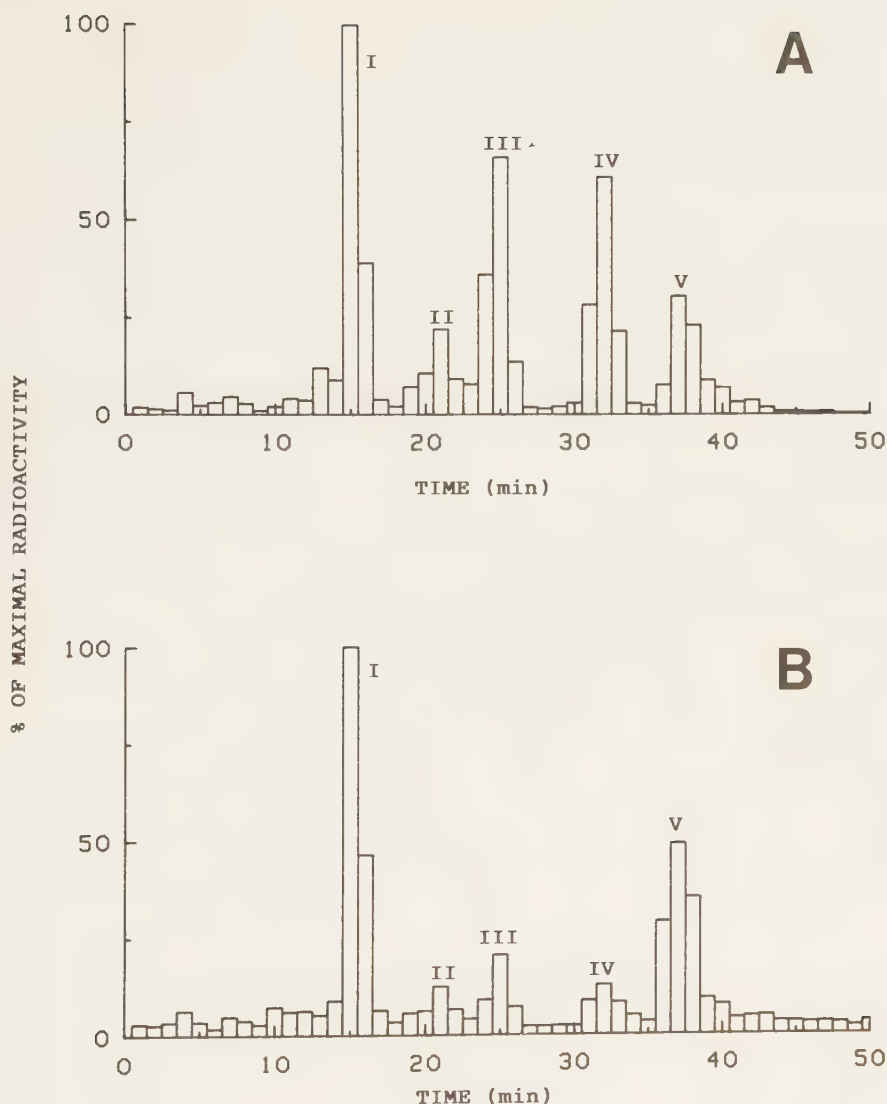


FIG.3. RADIOCHEMICAL ELUTION PROFILES OF TWO ETHYL ACETATE EXTRACTS CONTAINING BUDESONIDE METABOLITES.

A; incubation of [^3H]budesonide with human liver 9000g supernatant fraction for 1 h. **B**; human plasma sample taken 30 min after i.v. injection of [^3H]budesonide. The peaks correspond to 16 α -hydroxyprednisolone (I), 6 β -hydroxybudesonide (III) and budesonide (V). Peak II has been tentatively identified as 23-hydroxybudesonide and peak IV corresponds to metabolite III (see text).

Metabolic acetal splitting of budesonide

16 α -Hydroxyprednisolone was rapidly formed after incubation of (22R)-budesonide with liver 9000g supernatant from male and female rat, mouse and man (FIG.4). The metabolic pathway was quantitatively very pronounced in the mouse (more than 70% conversion for (22R)-budesonide) and in man (50% conversion), but not in the rat (5-10% and 15-20% conversion in female and male rat, respectively).

The extensive biotransformation of budesonide, giving 16 α -hydroxyprednisolone, proceeds by splitting of the acetal group. In GCs having the symmetric 16 α ,17 α -acetonide configuration, the acetal group is metabolically stable. Experiments were therefore undertaken to elucidate the splitting of the nonsymmetric acetal group of budesonide in detail.

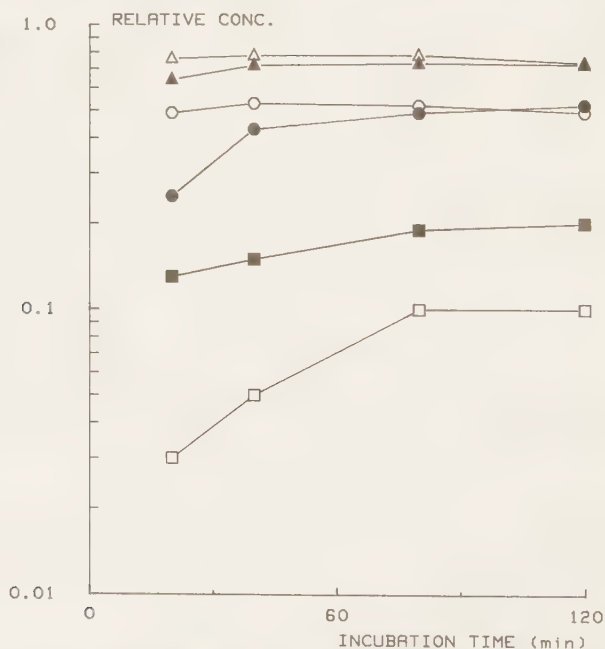


FIG.4. CONCENTRATION OF 16 α -HYDROXYPREDNISOLONE AFTER INCUBATION OF (22R)-BUDESONIDE WITH LIVER 9000g SUPERNATANT FRACTION FROM RAT (■,□), MOUSE (▲,△) AND MAN (●,○). Filled symbols indicate male and open symbols female livers. Initial concentration of the parent compound was set to 1.0.

When esterase inhibitors were added together with budesonide to liver 9000g supernatant fraction, no 16 α -hydroxyprednisolone could be detected. Instead a new compound, identified by mass spectrometry as 16 α -butyryloxy-prednisolone, was formed. Results from incubations performed under carbogen and ^{18}O -atmosphere, showed that atmospheric oxygen is incorporated at the 22-carbon of budesonide by a microsomal monooxygenase, i.e. cytochrome P-450. The resulting hypothetical product, 16 α -hydroxyprednisolone-16 α ,17 α -hemiortho-butyrate is expected to be chemically labile and will rearrange to the energetically more favourable 16 α -butyryloxy-prednisolone isomer. In the presence of liver esterase activity, 16 α -butyryloxy-prednisolone was instantaneously hydrolyzed to 16 α -hydroxyprednisolone and butyric acid. The suggested mechanism is illustrated in FIG.5.

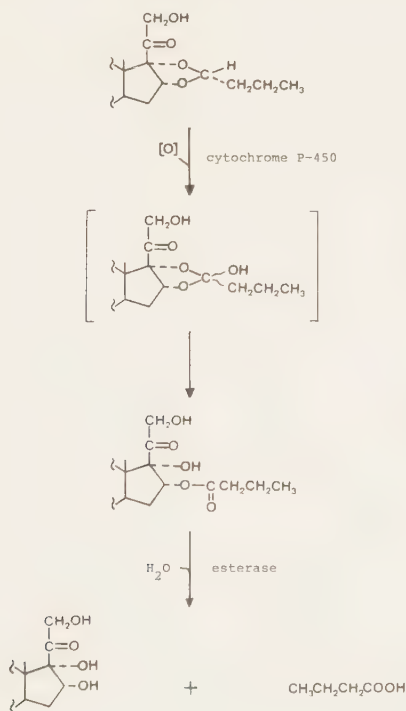


FIG.5. SUGGESTED MECHANISM FOR THE 16 α ,17 α -ACETAL SPLITTING OF (22R)-BUDESONIDE.

structure in brackets denote a postulated hemiorthoester intermediate.

Systemic elimination of budesonide in man; metabolic aspects

The metabolism of budesonide was investigated after i.v. administration of [^3H]budesonide (100 μg) to 4 healthy subjects. Individual plasma levels of total radioactivity, parent compound and quantitatively important metabolites are given in FIG.6. Plasma data did not indicate retention of any major pharmacologically active constituent. Both budesonide and the major metabolite, 16 α -hydroxyprednisolone, were rapidly eliminated from plasma. The similar plasma $t_{1/2}$'s (2.5 and 2.1 h for budesonide and 16 α -hydroxyprednisolone, respectively) indicate that the kinetics of 16 α -hydroxyprednisolone was formation rate limited.

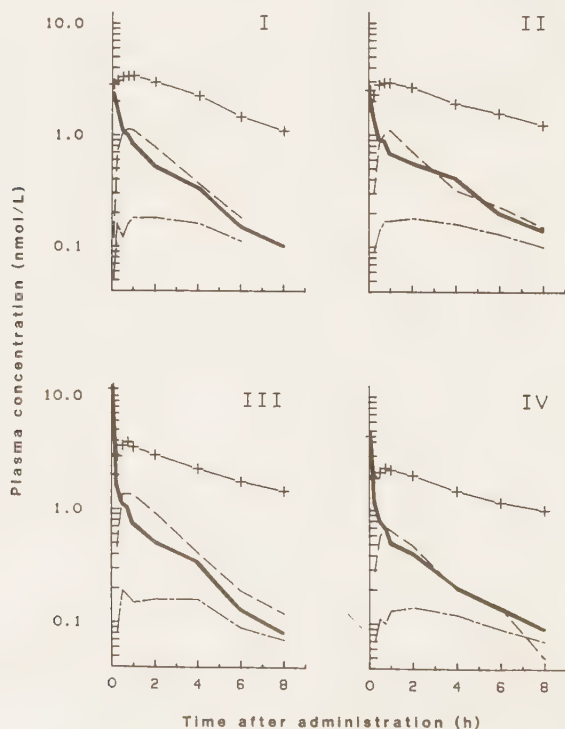


FIG.6. PLASMA LEVELS OF RADIOACTIVE CONSTITUENTS AFTER I.V. ADMINISTRATION OF 100 μg (230 nMOL) [^3H]BUDESONIDE TO SUBJECT I-IV.

+--++, total radioactivity; —, unchanged budesonide; - - -, 16 α -hydroxyprednisolone; - · - ·, 6 β -hydroxybudesonide.

The recovery of radioactivity in urine and faeces after i.v. administration was 57 and 34%, respectively, in 96 h. The major part of urinary radioactivity (82%) was excreted within 12 hours after administration. Of the 0-24 hour urine radioactivity, 34% could be accounted for as glucuronated and/or sulphated metabolites and 24% as 16 α -hydroxyprednisolone.

Urinary excretion rates of total radioactivity, 16 α -hydroxyprednisolone and 6 β -hydroxybudesonide from 0-24 h after budesonide administration are given in FIG.7. No or minimal amounts of unchanged budesonide was detected in any of the urine samples analyzed. The urinary excretion rate $t_{1/2}$ of 16 α -hydroxyprednisolone (2.9 h) agreed reasonably well with plasma $t_{1/2}$ of this compound (FIG.7) and the parent compound (see above).

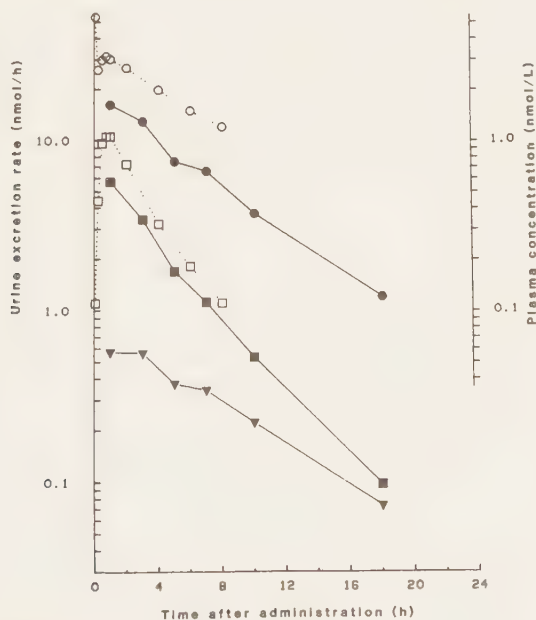


FIG.7. URINE EXCRETION RATES (SOLID LINES, FILLED SYMBOLS) AND PLASMA LEVELS (DOTTED LINES, OPEN SYMBOLS) OF RADIOACTIVE CONSTITUENTS AFTER I.V. ADMINISTRATION OF 100 μ g (230 nMOL) [3 H]BUDESONIDE TO MAN.

Values are mean of four subjects. ($\bullet$, $\circ$); total radioactivity, ($\blacksquare$, $\square$); 16 α -hydroxyprednisolone, ($\blacktriangledown$); 6 β -hydroxybudesonide. The same logarithmic scale was used for urine and plasma data.

Nasal bioavailability of budesonide in man

[³H]Budesonide (100 µg) was given by intravenous administration and nasal instillation to 4 healthy male subjects. After i.v. dosing, budesonide was rapidly eliminated from plasma. A high tissue uptake and a large systemic clearance resulted in a mean plasma $t_{1/2}$ of 2.5 h.

The nasal dose was instilled by a micro-pipette in order to minimize gastro-intestinal deposition. After nasal administration, T_{max} was approximately 30 min, and the systemic availability was $102 \pm 16\%$. FIG.8 shows the plasma levels of unchanged budesonide after the i.v. and nasal dosings. The intravenous parameters are summarized in TABLE 1.

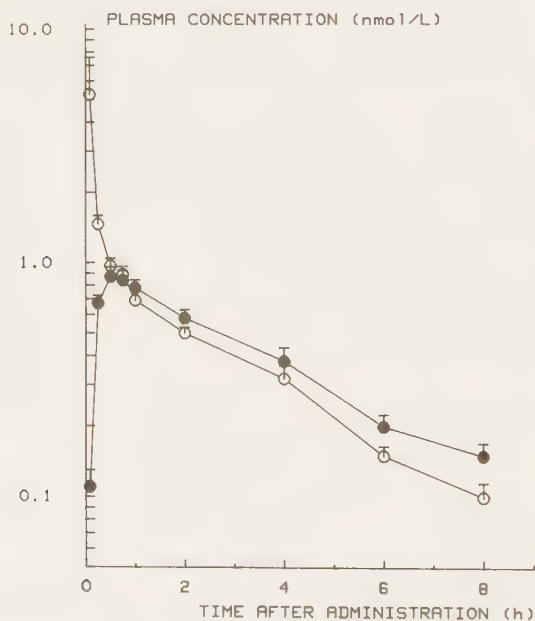


FIG.8. MEAN (+ SEM) PLASMA CONCENTRATION OF [³H]BUDESONIDE AFTER NASAL (●) AND I.V. (○) ADMINISTRATION OF 100 µg (230 nMOL, 50 µCi) TO 4 HEALTHY SUBJECTS.

TABLE 1. PHARMACOKINETICS OF [^3H]BUDESONIDE IN 4 HEALTHY MALE SUBJECTS GIVEN 100 μg INTRAVENOUSLY.

	Mean $\pm$ S.D.
CL (L/min)	0.92 ± 0.17
V_{β} (L/kg)	2.82 ± 0.48
$t_{1/2}$ (h)	2.53 ± 0.37

Pharmacokinetics of the budesonide epimers

The pharmacokinetics of the budesonide epimers were separately studied after i.v. administration of 500 μg [^3H]budesonide (the 1:1 epimeric mixture) to 6 healthy male subjects. The plasma $t_{1/2}$ of the two epimers was similar, but V_{β} and thus CL of

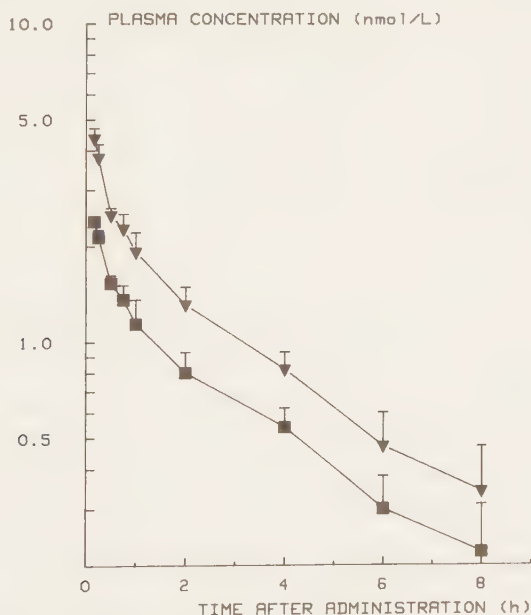


FIG.9. PLASMA CONCENTRATIONS (MEAN + SEM) OF EPIMERS 22S (▼) AND 22R (■) AFTER I.V. ADMINISTRATION OF [^3H]BUDESONIDE (500 μg , 1.16 μmol) TO 6 HEALTHY SUBJECTS.

epimer 22R was significantly larger ($p < 0.01$) than that of epimer 22S. FIG.9 shows the plasma levels of each epimer up to 8 h after administration. The basic pharmacokinetic parameters are summarized in TABLE 2.

Serum binding of (22R)-budesonide ($88.6 \pm 1.0\%$, mean $\pm$ S.D., $n=12$) was significantly lower ($p=0.0002$) than that of (22S)-budesonide ($90.7 \pm 1.5\%$). The serum free fraction (11.4 and 9.3% for epimers 22R and 22S) thus differed by approximately 20%.

TABLE 2. PHARMACOKINETICS OF [^3H]BUDESONIDE EPIMERS 22R AND 22S IN 6 HEALTHY MALE SUBJECTS GIVEN 500 μg INTRAVENOUSLY.

	Mean $\pm$ S.D.	
	22R	22S
CL (L/min)	1.95 ± 0.66	1.11 ± 0.32
V_{β} (L/kg)	5.98 ± 1.40	3.45 ± 0.54
$t_{1/2}$ (h)	2.66 ± 0.57	2.71 ± 0.69

Systemic effects after bolus doses of budesonide

After i.v. administration of 500 μg budesonide to 6 healthy subjects, systemic effects were measured as influence on plasma cortisol and white blood cell (WBC) counts. Plasma cortisol concentrations were slightly depressed by budesonide. A slight and transient decrease in total WBCs and lymphocytes was found as well as a slight increase in neutrophils. There were no statistically significant effects on plasma eosinophils.

The effects on plasma cortisol and WBC either after i.v. or nasal dosage of 100 μg budesonide were marginal.

Metabolism of glucocorticoids

Metabolism of cortisol

By 1965, the pathways of cortisol metabolism were in large parts elucidated (cf. Dorfman and Ungar 1965) and by 1977, Monder and Bradlow (Monder & Bradlow 1977 & 1980, Bradlow & Monder 1979) completed the picture by their work on corticoic and cortienic acid metabolites of cortisol. 11-Oxidoreduction, A-ring reduction and 17 β side chain oxidations are the major metabolic events of cortisol (FIG.10). Hydroxylation in 6 α - and 6 β -position do also occur to a minor extent, 6 β -hydroxycortisol being the major unconjugated cortisol metabolite in human urine (Frantz et al. 1961, Pal 1978). More than 90% of urinary radioactivity after an i.v. dose of radioactive cortisol consists of conjugated metabolites, the major fraction ($\approx 90\%$) being glucuronic acid conjugates (Kornel & Saito 1974, Gower 1975).

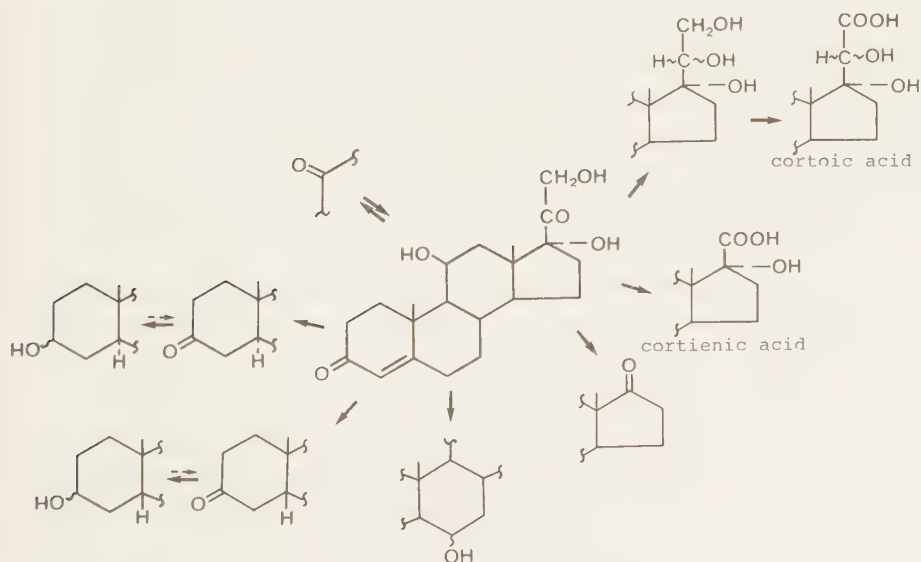


FIG.10. MAJOR METABOLIC PATHWAYS OF CORTISOL

Cortisol catabolism is located mainly to the liver, although other organs such as kidney, placenta, lung, muscle and skin may contribute to the metabolism of endogenous and synthetic GCs (Reach et al. 1977, Rocci et al., 1981, Hierholzer et al. 1982, Levitz et al. 1978, Blanford & Pearson Murphy 1977, Kolanowski et al. 1981, Hartiala 1976, Nicholas & Lugg 1982, Hsia 1971). The importance of intestinal microorganisms in the biotransformation of C₂₁-steroids is at present unclear (Gustafsson et al. 1984).

Metabolism of synthetic glucocorticoids, including budesonide

Potential of GC activity may partly be achieved by slowing down the rate of elimination, and this was the ultimate purpose of the initial research in this area (Glenn et al. 1957). The introduction of a Δ^1 -double bond in cortisol to give prednisolone, resulted in a lower rate of metabolism due to less extensive reduction of the Δ^4 -3-one group (Gray et al. 1956).

Metabolic pathways of synthetic GCs were reviewed in 1972 (cf Fotherby & James). TABLE 3 summarizes mainly later work. 6 β -Hydroxylation is an important metabolic pathway for many of the synthetic glucocorticoids. In general, A-ring reduction is less abundant than for cortisol, chiefly due to the Δ^1 -double bond (see above). Oxidation at C₂₁ to the corresponding acid has been reported for a few of the synthetic GCs and complete degradation of the C₂₀-C₂₁ side chain giving 17-keto- C₁₉ steroids has also been reported. Esterase activity are high in many tissues in the body (Junge 1977, Williams 1985), and hydrolysis are generally the primary metabolic event of GC esters. Data concerning phase II reactions with synthetic GCs are scarce and incomplete, but the conjugated fraction of urinary metabolites is generally less than that for cortisol (Araki et al. 1966, Lambe et al. 1978, Vermeulen 1959, Rice et al. 1974, Tredger et al. 1973, Chu et al. 1979, Chaplin et al. 1980a, Butter & Gray 1970, Miyabo et al. 1976).

TABLE 3 METABOLIC PATHWAYS OF SYNTHETIC GLUCOCORTICOIDS.

Metabolic pathway	Compound	Investigated species	References
11-oxidoreduction	Prednisone, prednisolone	man	Gray et al. (1956)
	Prednisolone	rat, man	Vermeulen (1958, 1959)
	Prednisone	mouse, dog, monkey	Eldareer et al. (1977)
	Prednisolone, dexamethasone, and betamethasone	man	Blanford et al. (1977)
	Dexamethasone	horse	Levitz et al. (1978)
	Betamethasone	man	Dumasia et al. (1979)
	Deflazacort	cynomolgus monkey	Butler & Gray (1970)
	Methylprednisolone	rabbit	Assandri et al. (1983)
6 β -Hydroxylation	Methylprednisolone-21-acetate	dog	Ehling et al. (1985)
			Buhler et al. (1965)
	Prednisone, prednisolone	man	Frey et al. (1984a)
	Dexamethasone	horse	Dumasia et al. (1979)
	Dexamethasone valerate	rat	Esumi et al. (1982)
	Desoxymethasone	rat, dog	Hornke et al. (1980)
	Deflazacort	rat, dog, man	Martinelli et al. (1979)
	Fluocortolone	man	Gerhards et al. (1971)
	Halcinonide	dog	Kripalani et al. (1977)
	Triamcinolone	dog	Florini et al. (1961a)
	Triamcinolone acetonide-21-phosphate	dog, monkey, rat	Kripalani et al. (1975)
	Triamcinolone acetonide	rabbit, dog, monkey, rat	Gordon & Morrison (1978)
	Betamethasone	man	Gerhards et al. (1971)
	Betamethasone 17-benzoate	rat	Tan et al. (1976)
	Flunisolide	mouse	Teitelbaum et al. (1981)
	Flunisolide	man	Tökes et al. (1981)
	Budesonide	man, mouse, rat	Edsbäcker et al. (I, II)
6 α -Hydroxylation	Deflazacort	cynomolgus monkey	Assandri et al. (1983)
	Fluocortolone	ram	Lambe et al. (1978)
6-Keto formation	Flunisolide	mouse	Teitelbaum et al. (1981)
7 α -Hydroxylation	Desoxymethasone	rat, dog	Hornke et al. (1980)
B-ring dehydrogenation	Deflazacort	rat, dog, man	Martinelli et al. (1979)
	Triamcinolone acetonide	rabbit, dog, rat, monkey	Gordon & Morrison (1978)
	Flunisolide	mouse	Teitelbaum et al. (1981)
	Budesonide	man, mouse, rat	Edsbäcker et al. (II)

continued on following page

TABLE 3 CONTINUED METABOLIC PATHWAYS OF SYNTHETIC GLUCOCORTICOIDS.

Metabolic pathway	Compound	Investigated species	References
21-Oxidation	Prednisolone	hamster	Lee (1977)
	Desoxymethasone	rat, dog	Horne et al. (1980)
	Fluocortolone	man	Gerhards et al. (1971)
	Halcinonide	dog	Kripalani et al. (1977)
	Triamcinolone acetonide	rabbit, dog, monkey, rat	Gordon & Morrison (1978)
	Beclomethasone-17 α ,21-dipropionate	rat	Oishi et al. (1981)
17 β -Side chain cleavage	Dexamethasone	horse	Skrabalak & Maylin (1982)
	Betamethasone	man	Butler & Gray (1970)
	Bethamethasone-17-benzoate	rat	Tan et al. (1976)
Acetal oxidation	Budesonide	man, mouse, rat	Edsbäcker et al. (I,II,III)
A-ring reduction	Prednisone, prednisolone	man	Gray et al. (1956)
	Prednisolone	rat, man	Vermeulen (1958, 1959)
	Fluocortolone	ram	Lambe et al. (1978)
	Deflazacort	rat, dog, man	Martinelli et al. (1979)
	Budesonide	man, mouse, rat	Edsbäcker et al. (II)
20-Reduction	Prednisone, prednisolone	man	Gray et al. (1956)
	Prednisone	man, rat	Vermeulen (1958, 1959)
	Prednisone	mouse, dog, monkey	El Dareer et al. (1977)
	Methylprednisolone-21-acetate	dog	Buhler et al. (1965)
	9 α F-cortisol	rat	Rafestin-Oblin et al. (1977)
	Dexamethasone	horse	Dumasia et al. (1979)
	Dexamethasone	rat	English et al. (1975)
	Dexamethasone valerate	rat	Esumi et al. (1982)
	Beclomethasone-17 α ,21-dipropionate	rat	Oishi et al. (1981)
	Betamethasone-17-benzoate	rat	Tan et al. (1976)
	Betamethasone	man	Butler & Gray (1970)
	Deflazacort	cynomolgus monkey	Assandri et al. (1983)
	Fluocortolon	man	Gerhards et al. (1971)
	Fluocortinbutylester	man	Mützel (1977)
Ester hydrolysis	Difluocortolonvaleriat	rat, guinea pig, man	Täuber & Toda (1976)
	Beclomethasone-17,21-dipropionate	man	Andersson & Ryrfeldt (1984)
	Beclomethasone-17,21-dipropionate and cyclomethasone	man, rat	Ronca-Testoni (1983a, 1983b)
	Beclomethasone-17,21-dipropionate and	rat	Nakano et al. (1981)
	Fluocortinbutylester	man	Mützel (1977)
	Various water-soluble glucocorticoid esters	man	Miyabo et al. (1976)
	Methylprednisolone acetate and betamethasone-17,21-dipropionate	man	Myers et al. (1982)
C ₂₁ -Decarboxylation	Fluocortinbutylester	man	Mützel (1977)

6 β -Hydroxylation, B-ring dehydrogenation and A-ring reductions are metabolic features of budesonide (II) common to other synthetic GCs. 11-Dehydrogenated budesonide metabolites were not found in the liver preparations investigated. Furthermore, no budesonide metabolite, biotransformed in the 17 β side chain, could be identified, neither in liver 9000g supernatant fraction from rat, mouse and man, nor in isolated perfused rat liver (S. Edsbäcker & E. Nilsson, unpublished observations). Reduction of the 20-keto group may be sterically hindered by substituents in 16-, 17- and 21-positions (Florini et al. 1961a, Lambe et al. 1978). The 16 α ,17 α -substituent in budesonide, like that in deflazacort (Martinelli et al. 1979), obviously inhibits biotransformation of both the 11-hydroxy- and the C₂₀-C₂₁ groups.

The symmetric 16 α ,17 α -isopropylidene substituent, present in many topical glucocorticoids, are metabolically stable (I, Kripalani et al. 1975, Chu et al. 1979). Two major metabolic pathways of budesonide involve the nonsymmetric 16 α ,17 α -acetal group, the acetal side chain hydroxylation giving 23-hydroxybudesonide (II) and the acetal side chain cleavage giving 16 α -hydroxyprednisolone (I,II,III). 16 α -Hydroxyprednisolone was formed only from (22R)-budesonide.

After i.v. administration of [³H]budesonide to healthy subjects, a similar metabolite pattern was observed as that observed in human liver preparations. Thus, 16 α -hydroxyprednisolone was abundant both in plasma and urine (Edsbäcker & Ryrfeldt 1986). Formed metabolites were rapidly excreted, predominantly in the urine, and to about one third as conjugates. Of the administered radioactivity, 53% was excreted in the urine within 24 hours. Assuming that half of the 0-24 h urinary radioactive recovery originated from (22R)-budesonide, 48% of this half could be accounted for as 16 α -hydroxyprednisolone. This yield was similar to that obtained in vitro in human liver (50%). The fact that the urinary levels of 16 α -hydroxyprednisolone were approximately 100-fold those of the parent compound in plasma (Edsbäcker & Ryrfeldt 1986), may form a non-invasive approach to estimate plasma t_{1/2} of budesonide.

Some metabolic structure-activity considerations

Certain structural elements are needed for retaining GC activity (Bush 1962). Briefly, reduction of the Δ^4 -double bond and oxidation of the 11-hydroxy group to the corresponding 11-keto compound strongly reduces biological activity. Reduction of the 20-keto group to a 20-hydroxyl configuration yields little, if any, biological activity, although exceptions have been reported (Lee & Soliman 1982). Glucocorticoid 17- and 20- carboxylic acids are generally inactive, but may retain topical antiinflammatory activity by esterification (Laurent et al. 1975, Gorsline et al. 1985). Glucocorticoid potency is furthermore diminished by B-ring hydroxylation or B-ring dehydrogenation (Bush 1962, Chaplin et al. 1980a). The major budesonide metabolites in man (I,IV) have strongly reduced glucocorticoid activities as compared to that of the parent compound (Dahlberg et al. 1984).

Glucocorticoid pharmacokinetics in man

Summary of basic kinetics

- Deposition and absorption. After nasal drug administration using pump sprays, a major fraction is deposited in the nasal cavity (Newman et al. 1986). After oral inhalation, a small portion may actually reach the lungs, the rest being swallowed (Newman 1985).

After administration of budesonide via the airways, peak plasma concentrations are reached within 30 min (IV, Ryrfeldt et al. 1982) indicating a high rate of absorption. A systemic availability of approximately 100 % after nasal instillation to healthy subjects suggests that there is no local biotransformation in the nose (IV). Budesonide is not metabolized in vitro in skin, lung or blood (Andersson & Ryrfeldt 1984). After oral inhalation of budesonide using the Inhaler^R tube, the systemic availability was 73% (after subtraction of the amounts deposited in the oral cavity, the tube and the actuator).

Gastro-intestinal absorption of GCs is considered to be extensive and fairly rapid (Duggan et al. 1975, Täuber et al. 1984, Gambertoglio et al. 1980, Martin et al. 1973, Derendorf et al. 1975), but for GCs with a high hepatic clearance such as budesonide (Ryrfeldt et al. 1982), the systemic availability is obviously limited by first pass elimination in the liver (cf Wilkinson & Shand 1975).

Budesonide, similar to other lipophilic GCs, is absorbed from human skin (Havu et al., unpublished observations, Täuber & Toda 1976, Mizuchi et al. 1976).

- Disposition. GC disposition data is summarized in TABLE 4. The literature dealing with prednisone and prednisolone pharmacokinetics was reviewed in 1980 (Gambertoglio et al. 1980). From the data presented in TABLE 4, it may be concluded that GCs generally have plasma $t_{1/2}$ in the order of a few hours, indicating a rapid systemic elimination. Plasma clearance and apparent volume of distribution varies however, the topically potent compounds (eg. triamcinolone acetate, flunisolide, budesonide) showing the highest values on these parameters.

FIG.11 shows the fraction of intravenously administered budesonide remaining in the body and in the plasma as unchanged compound (Data taken from paper IV). Approximately 90 % of administered drug has been eliminated within 8 hours. The extensive tissue distribution is indicated by the fact that more than 98 % of the body content of budesonide is located outside of plasma.

The liver is considered to be the major site of GC elimination, and no or small amounts of unchanged steroids are found in the urine (Tsuei et al. 1979, Fukushima et al. 1960, Möllman et al. 1985, Chaplin et al. 1980a, Peterson et al. 1960, Duggan et al. 1975, Gerhards et al. 1971, Martinelli et al. 1979, Derendorf et al. 1985). The urinary excretion of prednisolone was 11-24 % of administered dose (Rose et al. 1981b). No or minimal amounts of unchanged budesonide was found in the urine after i.v. administration of the drug in man (V, Edsbäcker & Ryrfeldt 1986). Thus,

TABLE 4 SUMMARIZED INTRAVENOUS KINETICS OF GLUCOCORTICOIDS IN MAN.

Compound	Dose	Subjects	t _{1/2} (h)	V (L/kg)	CL (L/min)	Comments ¹	Reference
Cortisol	100 mg	4F	1.8	1.42	-	morning values, psych. patients on maint. therapy with Haloperidol	Morselli et al. (1970)
	5,10,20,40 mg ³	6M	1.6	0.4	0.4	dose dep. kinetics ²	Toothaker & Welling (1983)
	tracer	8F,4M	1.07	-0.24	0.22	primarily convulsive patients	Choi et al. (1971)
	50-500 mg	20	1.9	-1	-		Peterson et al. (1955)
Prednisolone	5 mg	5M	2.8	0.49	0.14		Täuber et al. (1984)
	0.075,1.5 mg/kg	4M,4F	-	0.67 ^{SS}	0.20	dose dep. kinetics ²	Meffin et al. (1984b)
	40 mg	8M,5F	3.2	0.82 ^{SS}	0.20		Boekennoogen et al. (1983)
	20-80 mg	3F	2.6	0.91 ^B	0.23	asthmatic patients ²	McAllister et al. (1981b)
	20 mg	3F,4M	3.7	0.24	0.07	tuberculosis patients	Bergrem & Refvem (1982)
	tracer,0.15, 0.30 mg/kg ⁶	10	3.3	0.44	0.11	4 normal subjects and 6 osteoarthritic patients	Pickup et al. (1977)
	40 mg	8M	3.4	0.86 ^{SS}	-0.20		Rose et al. (1981a)
	0.8 mg/kg	10F	-	0.64 ^{SS}	0.17		Frey et al. (1984b)
	16.3 mg/kg	4M,3F	2.6	1.1 ^{SS}	0.18	bowel disease patients	Milsap et al. (1983)
Prednisone	10 mg	10	3.3	0.97	0.26		Shalm et al. (1977)
Methylprednisolone	1 mg/kg or 40 mg ³	4F,6M	2.5	1.13 ^{SS}	0.41	severe asthmatics	Szeffler et al. (1980)
	1 and 1.5 g ^{3,4}	4F	2.4	0.88	0.25	rheumatoid patients	Narang et al. (1983)
	10 mg/kg, 63 mg ^{4,5}	6F,11M	3.3	-1.12 ^B	0.31	dose dep. kinetics ²	Derendorf et al. (1985)
	2 mg	5F,8M	2.8	-0.87	0.27*		Stjernholm & Katz (1975)
	40 mg	14M	2.4	0.84 ^{SS}	0.27		Antal et al. (1983)
Dexamethasone	6.7 mg ^{4,6}	6F,6M	2.9	0.77 ^{SS}	0.25		Tsuei et al. (1979)
	1 mg ^{4,6}	2M	2.8	1.45 ^B	0.46		Kasuya et al. (1984)
	4 mg ^{4,6}	8M	4.5	1.08 ^{SS}	-0.21		Rose et al. (1981a)
	0.5 or 1.5 mg	4F,5M	4.2	0.61*	0.20*	3 hirsute, 1 epil. and 1 pept. ulcer patients	Haque et al. (1972)
	5,10 or 20 mg ⁶	6F,8M	3.0	1.15	0.47	patients during neurosurgery	McCafferty et al (1981)
Betamethasone	8 mg ^{4,6}	6F,2M	5.6	1.35 ^B	0.18		Petersen et al. (1983)
Deflazacort ⁷	50 mg	3M	2.9	-	0.14	values based on total radioactivity	Alessandro et al. (1980)
Fluocortolone	5 mg	5M	1.3	0.84	0.45		Täuber et al. (1984)
Fluocortinbutylester	1 mg	2M	2.5	-	-		Mützel (1977)
Flunisolide	1 mg	12M	1.6	1.8 ^B	0.96	Data based on whole blood levels	Chaplin et al. (1980a)
Triamcinolone acetonide	80 mg, 10 mg/kg ^{4,6}	8	1.5	1.88 ^B	0.96	dose dep. kinetics ²	Möllman et al. (1985)
Budesonide	0.5 mg	6M	2.8	4.3 ^B	1.4		Ryrfeldt et al. (1982)
	0.1 mg	4M	2.5	2.8 ^B	0.92		Edsbäcker et al. (IV)

 β = Apparent volume of distribution calculated using terminal rate constant

SS= " " " " " " moment analysis

* Two compartment data; V=volume of outer pool (V_2) and CL_p =metabolic clearance rate= $V_1 \cdot K_2$.

1= Included subjects were healthy and "normal" unless otherwise stated.

2= Values are mean in the investigated dosage interval.

3= Administration as the sodium succinate ester.

4= Dose based on the free alcohol.

5= Administration as the hemisuccinate ester.

6- Administration as the phosphate ester.

7= Oral data.

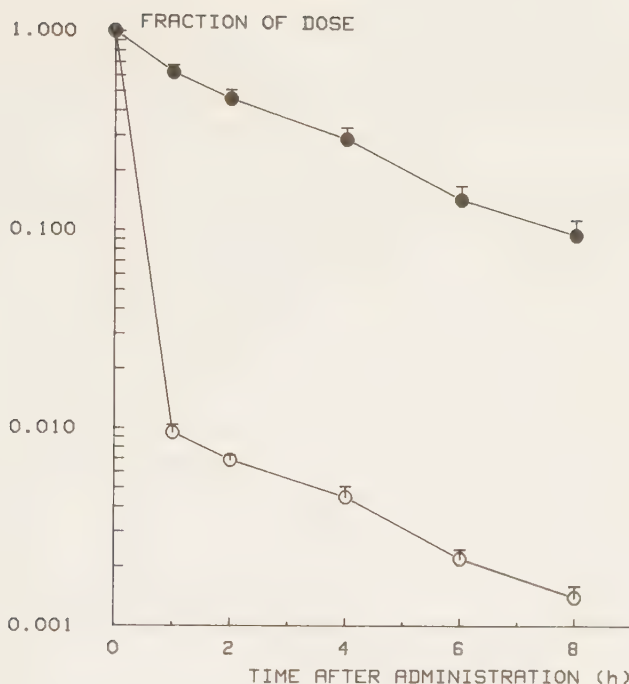


FIG.11. FRACTION OF ADMINISTERED BUDESONIDE REMAINING IN THE BODY (FILLED SYMBOLS) AND IN THE PLASMA (OPEN SYMBOLS) Values are mean + SEM of four subjects.

the plasma clearance of budesonide is high as a result of an extensive liver biotransformation (II, Ryrfeldt et al. 1982, Andersson 1982c). The oral availability is low (11-13%, Ryrfeldt et al. 1982, Borgå et al., unpublished observations) due to a high first pass elimination of the drug.

- Excretion. A major fraction of administered GCs generally ends up as urinary metabolites (Peterson et al. 1955, Gerhards et al. 1971, Butler & Gray 1970, Alessandro et al. 1980, Chaplin et al. 1980a, Mützel 1977, Fukushima et al. 1960, Vermeulen 1959, Brooks et al. 1972, Haque et al. 1972). After i.v. administration of [^3H]budesonide, 47% of the dose was excreted in the urine within 12 hours (IV). Within 96 h of dosage approximately 2/3 of recovered radioactivity after oral, orally inhaled and i.v. [^3H]budesonide is found in the urine (Ryrfeldt et al. 1982, Edsbäcker & Ryrfeldt 1986).

Disposition of budesonide epimers 22R and 22S

- In vitro aspects. An equation describing the relation between hepatic extraction ratio, blood flow and liver metabolizing capacity have been suggested by Gillette (1975) and Wilkinson & Shand (1975). Assuming complete intestinal absorption of unchanged drug, this formula allows for an estimation of oral availability from hepatic clearance data or vice versa. In an interesting work of Rane et al. (1977), hepatic extraction ratios (E) for several drugs were calculated from in vitro estimates of K_M , V_{max} and unbound drug fraction in blood. The in vitro estimates of E and oral availability ($F_o=1-E$) correlated well with the in vivo estimates of the corresponding parameters.

Budesonide epimer 22R is metabolized two to three times as rapid as epimer 22S in human liver preparations (Andersson et al. 1982c). In preliminary experiments, using the in vitro method of Rane et al. (1977), oral availabilities of 5% and 17% (mean from two livers) could be estimated for (22R)- and (22S)-budesonide respectively (S. Edsbäcker, unpublished results). This may be associated with systemic availabilities in man of epimer 22R and 22S of 8 and 18% respectively (Borgå et al., unpublished results).

- In vivo aspects. The disposition of budesonide epimer 22R differs from that of epimer 22S (V). The rapidly occurring divergence in plasma levels of budesonide epimer 22R as compared to epimer 22S show that there is a difference in distribution between the two epimers. The lung uptake of epimer 22R is 1.4 times larger than that of epimer 22S (Ryrfeldt et al., in manuscript), suggesting a higher tissue affinity for the former epimer. Interestingly, the GC receptor affinity of budesonide, being 200 times higher than that of cortisol, is twice as high for epimer 22R as for epimer 22S (Dahlberg et al. 1984).

Serum protein binding of many synthetic GCs is probably restricted to albumin (VI, Rousseau et al. 1972, Peets et al. 1969, Florini & Buyske 1961b, Ballard 1975). The higher serum free

fraction of (22R)- than of (22S)-budesonide explains in part the different volumes of distribution of the two epimers. The equation proposed by Øje & Tozer (1979) may be used to estimate the distribution of albumin bound drug, as well as the extra- and intra-cellular distribution of total drug (Rowland & Scheiner 1985). In TABLE 5, some interesting differences in distribution between the two epimers may be distinguished: Compared to epimer 22R, nearly twice as much of epimer 22S is bound to plasma and extracellular proteins. In addition, the distribution in extracellular fluids of epimer 22S exceeds that of epimer 22R. However, more than 95% of the two epimers present in the body is distributed intracellularly.

Plasma clearance of (22R)-budesonide is higher than that of (22S)-budesonide. Both epimers of budesonide may however be considered as high hepatic clearance compounds (Wilkinson & Shand 1975). Elimination is thus primarily limited by liver blood flow, rather than the metabolic activity of the liver

TABLE 5. DISTRIBUTION OF BUDESONIDE EPIMERS 22R AND 22S
ACCORDING TO THE EQUATION OF ØJE ($V=7+8fu+27fu/fu_p$)

Fraction of drug in body	Relationship	22R	22S
Unbound	$42fu/V$	0.011	0.016
Bound to proteins in serum	$3(1-fu)/V$	0.006	0.011
Bound to extracellular proteins	$7(1-fu)/V$	0.015	0.026
Bound intracellularly (in or on tissue cells, incl. blood cells)	$(V-7-35fu)/V$	0.974	0.958
In extracellular fluids	$(7+8fu)/V$	0.019	0.032
Outside extracellular fluids	$(V-(7+8fu))/V$	0.981	0.968

(Nies et al. 1976). Since the erythrocyte/plasma distribution ratio of budesonide is close to unity (Ryrfeldt et al. 1982), blood and plasma clearance is assumed to be equal. A blood clearance of 1.9 L/min for epimer 22R, being higher than normal liver blood flow (1.5-1.8 L/min, Boxenbaum 1980, Ganong 1981b), may imply that the calculated AUC of at least epimer 22R is underestimated. At 8 h after administration, approximately 15% of each epimer remains to be eliminated, possibly in part from a high affinity tissue site - a "deep compartment" not detected by the current analytical assays. Other clearance values of the budesonide epimers may be obtained if more sensitive analytical assays are used, or by evaluating the kinetics at steady state.

Factors regulating the disposition and metabolic inactivation of glucocorticoids

Species and sex differences

There are considerable differences between species in microsomal monooxygenase activity (cf Walker 1978), and species differences in GC disposition and metabolism have been reported (ElDareer et al. 1977, Chaplin et al. 1978). Many investigators have observed, especially in the rat, a sex-related difference in metabolism of drugs and hormones (cf Kato 1974), including steroids (Schrievers 1967). A sex-related difference in prednisolone disposition has been reported in man (Meffin et al. 1984b). Andersson et al. (1982d) have shown that the more rapid elimination of budesonide in male rat is reflected in lower systemic glucocorticoid effects in this sex.

The sex difference in budesonide metabolism in rats was associated with differences in oxidative capacity and different activities towards A-ring reduction (II). The disparity in budesonide metabolism between species and in the rat between sexes may have implications in the preclinical evaluation of the drug. From a metabolic point of view, the mouse was suggested to be a more relevant species than the rat when studying the pharmacology and toxicology of budesonide (II).

Drug interactions

Phenytoin, phenobarbital, rifampicin, primidone, ephedrine, phenylbutazone and thyroxine are examples of compounds which alter glucocorticoid disposition by inducing liver metabolic capacity (Gambertoglio et al. 1980, Schrievers 1967, Kupfer & Partridge 1970, Haque et al. 1972, Buffington et al. 1976, Jubiz et al. 1970, Kawai et al. 1985, Hancock & Level 1978, Stjernholm & Katz 1975, Brooks et al. 1972, 1976 & 1977, Choi et al. 1971, Conney et al. 1973, Petereit & Meikle 1977, Bergrem & Refvem 1982, Kuntzman et al. 1966). The metabolism of synthetic GCs may furthermore be accelerated after long term treatment (Araki 1966), and specific forms of rat liver cytochrome P450 are in fact induced by GCs (Schuetz 1984).

Of more concern in topical GC therapy may be agents which slows down the systemic elimination of steroidal drugs, hereby increasing the risk for GC side effects. Sex hormones and anabolic steroids (Schrievers 1967) as well as oral contraceptives (Kozower et al. 1974, Meffin et al. 1984a, Frey et al. 1984b, Boekenooen et al. 1983) may have this effect on GC disposition. Troleandomycin has a "steroid-sparing" effect on methylprednisolone but not on prednisolone, possibly due to selective inhibition of methylprednisolone metabolism (Szeffler et al. 1982). Cyclosporine inhibits prednisolone metabolism in kidney transplanted patients (Öst 1984, Langhoff et al. 1985).

Cimetidine is a commonly prescribed drug for GI ulcer disease. This H_2 -receptor blocking agent has shown to inhibit the systemic elimination of other drugs, especially those referred to as having a high hepatic clearance (Somogyi & Gugler 1982). Cimetidine and budesonide may be used simultaneously, e.g. in nocturnal asthma linked to reflux esophagitis (Shapiro & Christie 1983). Inhibition by cimetidine on the in vitro biotransformation rate of budesonide in human liver 9000g supernatant fraction is shown in TABLE 6. A 100-fold surplus of cimetidine caused at least 50% inhibition in budesonide biotransformation rate in vitro. In vivo, the effects will probably not be as marked, due to the high overall metabolizing capacity of

the liver. Furthermore, liver blood flow largely regulates the systemic elimination of budesonide. The difference in recommended daily dosage between the two drugs is about 1000 times and the clinical implications of an interaction may nevertheless be of importance.

TABLE 6. % INHIBITION OF CIMETIDIN (0.5 AND 2 mM) ON THE IN VITRO BIOTRANSFORMATION RATE OF BUDESONIDE IN HUMAN LIVER 9000g SUPERNATANT FRACTION.

Values are mean from experiments with two livers.

Budesonide concentration (μ M)	% Inhibition	
	Cimetidine conc (mM)	
	0.5	2.0
2	62	83
5	56	85
20	52	67

Diseased state

Asthma does not seem to influence prednisolone pharmacokinetics following oral dosage (McAllister et al. 1981a). Impaired renal and adrenal function have been reported to influence the metabolic clearance rate of GCs (Papen et al. 1982, Kawai et al. 1985, Gatti et al. 1984, Bergrem et al. 1985) but the reason for this remain unclear. Thyroid disease affects GC disposition, such that hyperthyroidism accelerates and hypothyroidism slows down the clearance of cortisol (Beisel et al. 1964, Jubiz & Meikle 1979).

Liver disease, including cirrhosis, may decrease the rate of GC elimination (Brown 1954, Peterson 1955 & 1960, Zumoff et al. 1967, Davies et al. 1978, Uribe 1977 & 1979, Kawai et al. 1985, Miyachi et al. 1979) and induce side effects (Kozower et al. 1974, Uribe et al. 1977). Altered serum protein binding following liver disease may also affect drug disposition (Blaschke 1977, Uribe et al. 1977). It may be speculated, that the pharmacokinetics of topical, "high hepatic clearance" GCs, such as budesonide, will be affected by liver disease, especially when considering that a major fraction of inhaled drug is swallowed and submitted to hepatic first pass metabolism (cf Blaschke & Rubin 1980).

Other factors

Pharmacokinetic polymorphism (cf Eichelbaum 1982), age (cf Woodhouse et al. 1984), altered hepatic blood flow (Gillette 1971), food intake (Schriefers 1967, Quigley et al. 1979, Barbhuiya & Welling 1982), body weight (Tsuei et al. 1979, Haque et al. 1972, Milsap et al. 1984) and extrahepatic metabolism (Tsuei et al. 1980) may theoretically influence GC pharmacokinetics. Smoking seems not to affect the pharmacokinetics of GCs (Rose et al. 1981a), although a stimulation of cortisol secretion has been shown in man (Kershbaum et al. 1968). Circadian variations in hepatic drug metabolism have been suggested (Radzialowski & Bousquet 1967, Jori et al. 1971) and this could possibly be one factor explaining the diurnal variations in plasma disappearance rate of prednisolone (Meffin et al. 1984b, English et al. 1983) and cortisol (DeLacerda et al. 1973, Morselli et al. 1970). Surgery and stress depress hepatic inactivation of GCs (Ganong 1981b). The dose-dependent pharmacokinetics of prednisolone, prednisone and cortisol is well documented (cf Rose et al. 1981b, Pickup et al. 1977, McAllister et al. 1981b, Toothaker & Welling 1982), and has been explained in terms of dose-dependent plasmaprotein-binding. Also triamcinolone acetonide and methylprednisolone may show dose dependent kinetics (Möllman et al. 1985, Derendorf et al. 1985).

Topical to systemic effect ratio - pharmacokinetic concepts

The rationale for administration of GCs by the topical route is that application of the drug directly to the diseased organ may reduce the dose needed for clinical efficacy, which in turn should decrease the incidence of systemic side effects. The first attempts to justify this concept were only partly successful; it could be demonstrated that local administration of cortisone (Gelfand 1951), cortisol (Foulds et al. 1955) and dexamethasone (Bickerman & Itkin 1963, Michels et al. 1967) was indeed effective. The treatment was however accompanied by more or less severe systemic side effects (Linder 1964, Novey & Beall 1965, Aaron & Muttitt 1965).

It has later been demonstrated that pulmonary inhaled drug doses to a large extent are swallowed (cf Newman 1985). The fraction of the swallowed drug which is absorbed and not inactivated during the first pass through the liver, will obviously be available for systemic activity. GC agents intended for systemic therapy, including those which were initially tested for topical efficacy, have a high bioavailability after oral administration (TABLE 7).

If it is assumed that GCs, applied topically in the respiratory tract, exert primarily a local effect (Kwaselow et al. 1985, Norman et al. 1966), and that furthermore liver biotransformation represents an inactivation process, then a low systemic availability should favour a high topical to systemic effect ratio. The systemic effects of flunisolide after various routes of administration closely correlated to the systemically available drug dose (Chaplin et al. 1980b). The availability of budesonide after oral administration in man is very low as compared to other GCs (TABLE 7). No data are presently available for beclomethasone-17 α ,21-dipropionate (BDP). Budesonide, being topically at least as potent as BDP, was however 2-4 times less potent systemically than BDP after oral administration of equivalent doses (Johansson et al. 1982).

TABLE 7. SYSTEMIC AVAILABILITY (F) OF GLUCOCORTICOIDS AFTER ORAL ADMINISTRATION IN MAN.

Drug	F (%)	Reference
Hydrocortisone	40-71	Toothaker et al. (1982)
Prednisolone	69-101	Gambertoglio et al. (1980)
	73-89	Täuber et al. (1984)
	99	Tanner et al. (1979)
	106	Al-Habet & Rogers (1980)
	60-66	Frey et al. (1984a)
	75-84	Rose et al. (1981a)
	82	Petereit & Meikle (1977)
Methylprednisolone	49-58	Narrang et al. (1983)
	99	Derendorf et al. (1985)
Dexamethasone	78-83	Duggan et al. (1975)
	53	Brophy et al. (1983)
	62	Kasuya et al. (1984)
	62-64	Rose et al. (1981a)
Fluocortolone	78-89	Täuber et al. (1984)
Flunisolide	21	Chaplin et al. (1980b)
BDP	unknown	
Budesonide	11	Ryrfeldt et al. (1982)
	13	Borgå et al. (unpublished)

The potential of GCs to cause effects on the formed elements of the blood as well as depression of plasma cortisol levels may be used as a sensitive index of GC side effects (cf Clissold & Heel 1984, Brogden et al. 1984, Pakes et al. 1980). Budesonide has a marginal effect on plasma cortisol levels and total and

differential white blood cell counts after single nasal (100 μ g) or i.v. (100 and 500 μ g) doses (V, VI). No or minimal effects on these parameters have been observed also after long term topical treatment with clinically recommended budesonide doses in asthmatic and rhinitic patients (cf Clissold & Heel 1984). This may, at least partly, be due to the high hepatic clearance and low oral availability of the drug.

The reason for the high clinical efficacy of budesonide is beyond the scope of this thesis. However, some recent and controversial work concerning the postulated topical effect of GCs may have pharmacokinetic implications. In papers of Norman et al. (1966) and Kwaselow et al. (1985), the therapeutic effect of dexamethasone and flunisolide in rhinitis was higher after nasal than after oral administration of systemically equivalent doses. This was taken as evidence for a pure topical effect of the two GCs. In an interesting paper of Brattsand et al. (1985b), it was demonstrated in an in vivo rat model that a similar anti-edema effect was obtained in both lungs after budesonide instillation in only one of the lungs. Furthermore, a similar anti-edema effect was obtained after i.v. as after i.t. administration of budesonide in rats. The authors suggested that the rapid systemic absorption, giving high plasma peak levels of unchanged compound, rather than a pure topical effect was responsible for the obtained results. Systemic side effects, measured as decreased adrenal weight, were suggested to be more dependent on persistent plasma levels of unchanged compound (Brattsand et al. 1985b). This concept, originally proposed by Harter et al. (1963), may explain the achieved dissociation between therapeutic effects and some or all of the obtained side effects.

To extend this hypothesis further, the pharmacokinetic data on budesonide presented here, may suggest that the systemically induced lung effects of budesonide are due to an extensive tissue distribution. The high tissue uptake of the drug has been specifically demonstrated in experiments with isolated perfused rat and guinea pig lungs (Ryrfeldt et al., in manuscript). After administration via the airways, budesonide

is rapidly absorbed into the systemic circulation (IV, Ryrfeldt et al. 1982). Following absorption, the drug will readily escape the blood and may redistribute into high affinity tissue sites, possibly including sites in the respiratory tract. Low blood levels should restrain any effect on blood cells. The brain/blood distribution ratio of budesonide in the mouse and the rat is less than 0.5 (Andersson & Edsbäcker, unpublished results). A similar or smaller ratio in man could explain the marginal effects of the drug on the HPA-axis and thus plasma cortisol levels.

To conclude, the favourable ratio between therapeutic and untoward effects noted for several topically potent GCs, can partly be explained by pharmacokinetic principles. Future research may clarify the basic concepts and unfold the ultimate reason behind the achieved selectivity.

SUMMARY AND CONCLUSION

The metabolic fate and some pharmacokinetic properties of budesonide have been elucidated. The drug, being a topically potent glucocorticoid with low systemic side effects, is a mixture of two epimers (22R and 22S) having the same pharmacological effect profile but having different intrinsic potencies.

Budesonide was extensively biotransformed in human, mouse and rat liver 9000g supernatant fraction to metabolites of low glucocorticoid activity. The major metabolic pathway of budesonide in human liver, the 16 α ,17 α -acetal splitting, is unique within the group of topically potent glucocorticoids. This biotransformation did proceed via an intermediary ester, giving 16 α -hydroxyprednisolone and butyric acid. It was substrate selective for epimer 22R. Other metabolic pathways did include 6 β -hydroxylation, Δ^6 -dehydrogenation, A-ring reduction and acetal side chain hydroxylation.

Species differences were observed in the metabolism of budesonide in rat, mouse and human livers. In the rat, there was a large sex difference. Of the two animal species studied, the liver biotransformation in the mouse was most similar to that of man.

The in vitro data obtained in liver preparations correlated well with in vivo results; the same major metabolites, formed after incubation of budesonide with human liver 9000g supernatant fraction, were present in plasma after intravenous administration in man.

Budesonide was readily absorbed from the respiratory tract, with no or negligible local biotransformation. After systemic uptake, the drug was extensively distributed into tissues. During the elimination phase, more than 98% of the body content of budesonide was located outside of plasma. The elimination was nevertheless rapid, due to a high hepatic clearance.

The budesonide epimers had different pharmacokinetic and metabolic properties. Both the apparent volume of distribution and plasma clearance of epimer 22S was significantly less than the corresponding values of epimer 22R. Different serum binding and liver biotransformation rates were contributing factors to this disparity between the epimers.

Topical glucocorticoids, including budesonide, are characterized by an extensive liver biotransformation. Factors to be considered in prolonged treatment with topical glucocorticoids include liver disease and agents, which may slow down the systemic elimination and which may hereby increase the incidence of glucocorticoid side effects.

In conclusion, the present study may offer metabolic and pharmacokinetic explanations for the clinical observation that budesonide can be administered via topical routes in therapeutically effective doses without inducing unwanted systemic glucocorticoid effects.

ACKNOWLEDGEMENTS

I want to express my deepest gratitude to my supervisor, Dr Åke Ryrfeldt, who introduced me to pharmacokinetics and who taught me most of what I know about glucocorticoids. His never failing support, encouragement and enthusiasm throughout this study has been invaluable to me. Tack för allt, Åke!

I am particularly indebted to

- Professor Karl-Erik Andersson, for his generous help and support, and for fruitful discussions.
- Arne Thalén, for providing crucial reference compounds and for sharing his deep knowledge of glucocorticoid chemistry.
- Paul Andersson, who introduced me to experimental pharmacokinetics. Thank you Paul, for sharing your knowledge, your friendship and my hardships!
- Dr Claes Lindberg, for his tenacious pursuit to obtain and interpret MS-data, for constructive criticism and endless patience. Thanks Claes for your stubbornness!
- Jan Paulsson, for his ingenious accomplishments concerning the LC-MS technique.
- To my other co-authors, Professor Romain Pauwels, Sven Jönsson and Kristina Geelmuyden for their contribution.

I am also indebted to

- Dr Olof Borgå, head of the Pharmacokinetics Laboratory, for his good advice and valuable criticism.
- Dr Lars Nyberg, for his friendly encouragement during the termination of this study.
- Associate Professor Ralph Brattsand for stimulating discussions on glucocorticoid pharmacology.
- Eva Nikander, Elin Owman and Pia Magnusson for patient secretarial help with the individual manuscripts. Eva Nikander also taught me to cope with the word processor.

I am grateful to the management of AB Draco for first-rate working conditions, and to all my colleagues at the Pharmacokinetic Laboratory for a pleasant atmosphere.

There are above all two persons whose contribution has been invaluable to me. Eva and Lina, I am sorry about all the time I have stolen from You!

REFERENCES

- Aaron TH, & Muttitt ELC (1965). Effects of intranasal dexamethasone phosphate on the adrenal function of patients with perennial rhinitis. *Ann Allergy* 23, 100-102.
- Agrup G, Björnberg A, Elmros T, Groth O, Hannuksela M, Lassus A, Salde L, Skogh M, & Thomsen K (1981). Clinical trial of a potent nonhalogenated topical steroid, budesonide. *Acta Dermatovenereol* 61, 180-182.
- Alessandro A, Antonio P, Guiseppe B, & Valeria P (1980). Disposition of a new steroidal anti-inflammatory agent, deflazacort, in rat, dog and man. *Eur J Drug Metab Pharmacokin* 5, 207-215.
- Al-Habet S, & Rogers HJ (1980). Pharmacokinetics of intravenous and oral prednisolone. *Br J Clin Pharmacol* 10, 503-508.
- Andersson P, & Brattsand R (1982a). Protective effects of the glucocorticoid, budesonide, on lung anaphylaxis in actively sensitized guinea-pigs: Inhibition of IgE- but not of IgG-mediated anaphylaxis. *Br J Pharmacol* 76, 139-147.
- Andersson P, Brattsand R, Bange C, Källström L, & Stahre G (1982b). Protective effects of budesonide on lung anaphylaxis in actively sensitized guinea-pigs. Inhibition of "IgE"-mediated anaphylaxis. *Eur J Respir Dis* 63, suppl 122, 263-265.
- Andersson P, Edsbäcker S, Ryrfeldt, Å, & von Bahr C (1982c). In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J Steroid Biochem* 16, 787-795.
- Andersson P, Brattsand R, Edsbäcker S, Källström L & Ryrfeldt Å (1982d). Biotransformation rate (in vitro) and systemic potency (in vivo) of the topical glucocorticoid budesonide in male and female rats. *J Steroid Biochem* 17, 703-706.
- Andersson P, & Ryrfeldt Å (1984). Biotransformation of the topical glucocorticoids budesonide and beclomethasone 17 α ,21-dipropionate in human liver and lung homogenate. *J Pharm Pharmacol* 36, 763-765.
- Antal EJ, Wright CE, Gillespie WR, & Albert KS (1983). Influence of route of administration on the pharmacokinetics of methylprednisolone. *J Pharmacokin Biopharm* 11, 561-576.
- Araki Y, Yokota O, Kato T, Kashima M, & Miyazaki T (1966). Dynamics of synthetic corticosteroids in man. In *Steroid dynamics*, eds Pincus G, Nakao T, & Tait JF, pp 462-480. New York: Academic Press.
- Assandri A, Ferrari P, Perazzi A, Ripamonti A, Tuan G & Zerilli L (1983). Disposition and metabolism of a new steroidal anti-inflammatory agent, deflazacort, in cynomolgus monkeys. *Xenobiotica* 13, 185-196.

Ballard PL (1975). Delivery and transport of glucocorticoids to target cells. *Monogr Endocrinol* 12, 25-48.

Barbahaiya RH, & Welling PG (1982). Influence of food on the absorption of hydrocortisone from the gastrointestinal tract. *Drug-Nutrient Interactions* 1, 103-112.

Baxter JD, & Rousseau GG, (1979). Glucocorticoid hormone action; An overview. In *Glucocorticoid hormone action*. Ed Baxter JD, & Rousseau GG, pp 1-24, Heidelberg: Springer-Verlag Berlin.

Beisel WR, Col LT, Di Raimondo VC, & Forsham PH, (1964). Cortisol transport and disappearance 60, 641-652.

Bergrem H, & Refvem OK (1982). Changes in prednisolone pharmacokinetics and protein binding during treatment with rifampicin. *Proc EDTA* 19, 552-557.

Bergrem H, Jervell J, & Flatmark A (1985). Prednisolone pharmacokinetics in cushingoid and non-cushingoid kidney transplant patients. *Kidney Int* 27, 459-464.

Bickerman HA, & Itkin SE, (1963). Aerosol steroid therapy and chronic bronchial asthma. *JAMA* 184, 533-538.

Blanford AT, & Pearson Murphy BE (1977). In vitro metabolism of prednisolone, dexamethasone, betamethasone and cortisol by the human placenta. *Am J Obstet Gynecol* 127, 264-267.

Blaschke TF (1977). Protein binding and kinetics of drugs in liver diseases. *Clin Pharmacokin* 2, 32-44.

Blaschke TF & Rubin PC (1980). Hepatic first pass metabolism in liver disease. *Clin Pharmacokin* 4, 423-432.

Boekenoogen SJ, Szeffler SJ & Jusko WJ (1983). Prednisolone disposition and protein binding in oral contraceptive users. *J Clin Endocrinol Metab* 56, 702-709.

Boxenbaum H (1980). Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. *J Pharmacokin Biopharm* 8, 165-176.

Bradlow HL, & Monder C (1979). Steroid carboxylic acids. In *Steroid Biochemistry*, ed Hobkirk R, pp 29-82, Fla: CRL press.

Brattsand R, Thalén A, Roempke K, Källström L, & Gruvstad E (1982a). Influence of 16 α ,17 α -acetal substitution and steroid nucleus fluorination on the topical to systemic activity of glucocorticoids. *J Steroid Biochem* 16, 779-786.

Brattsand R, Andersson P, Wieslander E, Linden M, Axelsson B, & Paulsson LI (1985a). Pathophysiological characteristics of a guinea-pig model for dual bronchial obstruction. In Glucocorticoids, inflammation and bronchial hyperactivity. Symposium at the 3rd congress of the European Society of Pneumology, Basel, sept 19, 1984. Ed Hogg JC, Ellul-Micallef R, & Brattsand R, pp 51-65 Amsterdam: Excerpta Medica.

Brattsand R, Källström L, Johansson U, & Dahlbäck M (1985b). Route of administration and rapid inactivation as determinants of the lung-specific actions of glucocorticosteroids. In Glucocorticoids, inflammation and bronchial hyperactivity. Symposium at the 3rd congress of the European Society of Pneumology, Basel, sept 19, 1984. Ed Hogg JC, Ellul-Micallef R, & Brattsand R, pp 145-153, Amsterdam: Excerpta Medica.

Brogden RN, Heel RC, Speight TM, & Avery GS (1984). Beclomethasone dipropionate. A reappraisal of its pharmacodynamic properties and therapeutic efficacy after a decade of use in asthma and rhinitis. *Drugs* 28, 99-126.

Brooks SM, Werk EE, Ackerman SJ, Sullivan I, & Thrasher K (1972). Adverse effects of phenobarbital on corticosteroid metabolism in patients with bronchial asthma, *N Engl J Med* 286, 1125-1128.

Brooks PM, Buchanan WW, Grove M, & Downie WW (1976). Effects of enzyme induction on metabolism of prednisolone. *Ann Rheum Dis* 35, 339-343.

Brooks SM, Sholiton LJ, Werk EE Jr, & Altenau P (1977). The effects of ephedrine and theophylline on dexamethasone metabolism in bronchial asthma. *J Clin Pharmacol* 17, 308-318.

Brophy TROR, Mc Cafferty J, Tyrer JH, & Eadie MJ (1983). Bioavailability of oral dexamethasone during high dose steroid therapy in neurological patients. *Eur J Clin Pharmacol* 24, 103-108.

Brown H, Williardson DG, Samuels LT, & Tyler FH (1954). 17-Hydroxycorticosteroid metabolism in liver disease. *J Clin Invest* 33, 1524-1532.

Buffington GA, Dominguez JA, Piering WF, Hebert LA, Kauffman M, & Lemann J (1976). Interaction of Rifampin and glucocorticoids. Adverse effect on renal allograft function. *JAMA* 236, 1958-1960.

Buhler DR, Thomas RC, & Schlagel, CA (1965). Absorption, metabolism and excretion of 6 α -methylprednisolone-3-H,21-acetate following oral and intramuscular administrations in the dog. *Endocrinology* 76, 852-864.

Bush IE (1962). Metabolic factors affecting the biological activities of steroid hormones. *Pharm Rev* 14, 361-401.

Butler J, & Gray CH (1970). The metabolism of betamethasone. *J Endocr* 46, 379-390.

Carrier HM, Koelsche GA, Prickman LE, Maytum CK, Lake CF, & Williams HL (1950). The effect of cortisone on bronchial asthma and hay fever occurring in subjects sensitive to ragweed pollen. *J Allergy* 21, 282-287.

Case DE, (1981). The state of the art; a commentary on the current practice of metabolism and pharmacokinetic studies in the pharmaceutical industry. *Xenobiotica* 11, 803-814.

Chaplin M, Matin S, Tökés L, Rooks II W, Swenson E, Maddox M, Chu N, Amos B, & Bell J (1978). Species variation in the first-pass metabolism of orally administered flunisolide (abstract). *Proc 7th Int Cong Pharmacol*. Pergamon Press, Oxford, Abst 2073.

Chaplin MD, Rooks II W, Swenson EW, Cooper WC, Nerenberg C, & Chu NI (1980a). Flunisolide metabolism and dynamics of a metabolite. *Clin Pharmacol Ther* 27, 402-413.

Chaplin MD, Cooper WC, Segre EJ, Oren J, Jones RE, & Nerenberg C (1980b). Correlation of flunisolide plasma levels to eosinopenic response in humans. *J Allergy Clin Immunol* 65, 445-453.

Choi Y, Thrasher K, Werk EE, Sholiton LJ, & Olinger C (1971). Effect of diphenylhydantoin on cortisol kinetics in humans. *J Pharmacol Exp Ther* 176, 27-34.

Chu NI, Amos BA, Tökés L, Maddox ML, Matin SB, Hama KM, Patterson JW, Wagner PJ, Bell JP, & Chaplin MD (1979). Disposition of flunisolide in the rat, mouse, dog, rhesus, monkey and cynomolgus monkey. *Drug Metab Disp* 7, 81-89.

Clark CR (1984). The cellular distribution of steroid hormone receptors: have we got it right? *Trends Biochem Sci* 9, 207-208.

Clissold SP, & Heel RC (1984). Budesonide - A preliminary review of its pharmacodynamic properties and therapeutic efficacy in asthma and rhinitis. *Drugs* 28, 485-518.

Conney AH, Levin W, Jacobson M, & Kuntzman R (1973). Effects of drugs and environmental chemicals on steroid metabolism. *Clin Pharmacol Ther* 14, 727-741.

Dahl R, & Johansson SÅ (1982). Importance of duration of treatment with inhaled budesonide on the immediate and late bronchial reaction. *Eur J Respir Dis* 63, suppl 122, 167-175.

Dahlberg E, Thalén A, Brattsand R, Gustafsson JÅ, Johansson U, Roempke K, & Saartok T (1984). Correlation between chemical structure, receptor binding, and biological activity of some novel, highly active, 16 α ,17 α -acetal substituted glucocorticoids. *Mol Pharmacol* 25, 70-78.

Dahlbäck M, Bergstrand H, & Brattsand R (1986). Antigen-induced bronchial anaphylaxis in actively sensitized SD rats: Effects of glucocorticoid treatment. *Allergy* (In press).

Davies M, Williams R, Chakraborty J, English J, Marks V, Ideo G, & Tempini (1978). Prednisone or prednisolone for the treatment of chronic active hepatitis? A comparison of plasma availability. *Br J Clin Pharmacol* 5, 501-505.

DeLacerda L, Kowarski A, & Migeon CJ (1973). Diurnal variation of the metabolic clearance rate of cortisol. Effect on measurement of cortisol production rate. *J Clin Endocrinol Metab* 36, 1043-1049.

Derendorf H, Möllmann H, Rohdewald P, Rehder J, & Schmidt EW (1985). Kinetics of methylprednisolone and its hemisuccinate ester. *Clin Pharmacol Ther* 37, 502-507.

Dorfman RI, & Ungar F (1965). Catabolic reactions of the steroids. In *Metabolism of steroid hormones* pp 289-381. New York: Academic Press.

Drayer DE (1982). Pharmacologically active metabolites of drugs and other foreign compounds. Clinical, pharmacological, therapeutic and toxicological considerations. *Drugs* 54, 519-542.

Duggan DE, Yeh KC, Matalia N, Ditzler CA, & McMahon FG (1975). Bioavailability of oral dexamethasone. *Clin Pharmacol Ther* 18, 205-209.

Dumasia MC, Houghton E, Moss MS, & Chakraborty J (1979). Metabolism of dexamethasone in the horse. *Proc 3rd Int Symp Equine Medication Control, Lexington, Kentucky*, 247-252.

Ebling WF, Szefer SJ, & Jusko WJ (1985). Methylprednisolone disposition in rabbits. Analysis, prodrug conversion, reversible metabolism, and comparison with man. *Drug Metab Disp* 13, 296-304.

Edsbäcker S, & Ryrfeldt Å (1986). Systemic elimination of the topical glucocorticoid budesonide in man; metabolic aspects. In manuscript.

Eichelbaum M (1982). Defective oxidation of drugs. Pharmacokinetic and therapeutic implications. *Clin Pharmacokin* 7, 1-22.

Eldareer SM, Struck RF, White VM, Mellett LB, & Hill DL (1977). Distribution and metabolism of prednisone in mice, dogs and monkeys. *Cancer Treatment Reports* 61, 1279-1289.

English J, Chakraborty J, & Marks V (1975). The metabolism of dexamethasone in the rat. Effect of phenytoin. *J Steroid Biochem* 6, 65-68.

English J, Dunne M, & Marks V (1983). Diurnal variations in prednisolone kinetics. *Clin Pharmacol Ther* 33, 381-385.

Esumi Y, Ohtsuki T, Alsumi K, Katami Y, Kato T, Yokoshima T, & Takahara Y (1982). Studies on the metabolic fate of dexamethasone-17-valerate. III Metabolites in rat. *Iyakuin Kenkyu* 13, 1037-45. *Chemical Abstracts* 98:47157 v.

- Farr RS (1985). One definition of asthma. *Chest* 87, 205-215.
- Fauci AS (1976). Glucocorticosteroid therapy: Mechanisms of action and clinical considerations. *Ann Int Med* 84, 304-315.
- Florini JR, Smith LL, & Buyske DA (1961a). Metabolic fate of a synthetic corticosteroid (triamcinolone) in the dog. *J Biol Chem* 236, 1038-1042.
- Florini JR, & Buyske DA (1961b). Plasma protein binding of triamcinolone-3H and hydrocortisone-4-¹⁴C. *J Biol Chem* 236, 247-251.
- Forsberg K, Ryrfeldt Å, & Sörenby L (1982). Protective effects of budesonide on lung anaphylaxis in actively sensitized guinea-pigs. Inhibition of "IgG"-mediated anaphylaxis. *Eur J Respir Dis* 63, suppl 122, 257-259.
- Fotherby L, & James F (1972). Metabolism of synthetic glucocorticoids. In *Advances in steroid Biochemistry and Pharmacology*, vol 3, ed Briggs MH, & Christie GA, pp 67-165, New York: Academic Press.
- Foulds WS, Greaves DP, Herxheimer H & Kingdom LG (1955). Hydrocortisone in treatment of allergic conjunctivitis, allergic rhinitis, and bronchial asthma. *Lancet* 1, 234-235.
- Frantz AG, Katz FH, & Jailer JW (1961). 6 β -Hydroxycortisol and other polar corticosteroids: measurement and significance in human urine. *J Clin Endocrinol Metab* 21, 1290-1303.
- Frey BM, Brandenberger AW, Frey FJ, & Widmer HR (1984a). Systemische Verfügbarkeit von Prednisolon nach oral verabreichtem Prednison. *Pharm Acta Helv* 59, 301-309.
- Frey BM, Schaad HJ, & Frey FJ (1984b). Pharmacokinetic interaction of contraceptive steroids with prednisone and prednisolone. *Eur J Clin Pharmacol* 26, 505-511.
- Fritz KA, & Weston WL (1983). Topical glucocorticosteroids - a review. *Ann Allergy* 50, 68-76.
- Fukushima DK, Bradlow HL, Hellman L, Zumoff B, & Gallagher TF (1960). Metabolic transformation of hydrocortisone-4-¹⁴C in normal men. *J Biol Chem* 235, 2246-2252.
- Gambertoglio JG, Amend WJC Jr, & Benet LZ (1980). Pharmacokinetics and bioavailability of prednisone and prednisolone in healthy volunteers and patients. A review. *J Pharmacokin Biopharm* 8, 1 - 52.
- Ganong WF (1981a). Endocrine functions of the pancreas & regulation of carbohydrate metabolism, and The adrenal medulla & adrenal cortex. In *Review of medical physiology*. 10th edition pp 264-307. Los Altos: Lange medical publications.

Ganong WF (1981b). Circulation through special regions. In Review of medical physiology. 10th edition p 476. Los Altos: Lange medical publications.

Garattini S (1983). Importance of establishing the presence of drug active metabolites - review paper. Eur J Drug Metab Pharmacokin 8, 97-108.

Gatti G, Perucca E, Frigo GM, Notarangelo LD, Barberis L, & Martini A (1984). Pharmacokinetics of prednisone and its metabolite prednisolone in children with nephrotic syndrome during the active phase and in remission. Br J Clin Pharmacol 17, 423-431.

Gelfand ML (1951). Administration of cortisone by the aerosol method in the treatment of bronchial asthma. N Engl J Med 245, 293-294.

Gerhards E, Neuweboer B, Schultz G, Gibian H, & Hecker W (1971). Stoffwechsel von 6 α -fluoro-16 α -methylpregna-1,4-dien-11,21-diol-3,20-dion (fluocortolon) beim Menschen. Acta Endocr 68, 98-126.

Gillette JR (1971). Factors affecting drug metabolism. Ann NY Acad Sci 179, 43-66.

Glenn EM, Stafford RO, Lyster SC, & Fowman BJ (1957). Relation between biological activity of hydrocortisone analogues and their rates of inactivation by rat liver enzyme systems. Endocrinology 61, 128-142.

Gordon S, & Morrison J (1978). The metabolic fate of triamcinolone acetonide in laboratory animals. Steroids 32, 2272-2284.

Gorsline J, Bradlow HL, & Sherman MR (1985). Triamcinolone acetonide 21-oic acid methyl ester: A potent local antiinflammatory steroid without detectable systemic effects. Endocrinology 116, 263-273.

Gower DB (1975). Catabolism and excretion of steroids. In Biochemistry of steroid hormones. Ed Makin HLJ, pp 149-185, London, Blackwell Scientific Publications.

Gray CH, Green MAS, Holness NJ, & Lunnon JB (1956). Urinary metabolic products of prednisone and prednisolone. J Endocrin, 14, 146-154.

Gruvstad E, & Bergtsson B (1980). A comparison of a new steroid, budesonide, with other topical corticosteroids in the vasoconstriction assay. Drugs Exptl Clin Res 6, 385-390.

Gustafsson JÅ, Rafter J, & Gustafsson B (1984). Kost-tarmfloracancer - bakteriernas roll vid omsättningen av endogena och exogena ämnen. Läkartidningen 81, 4015-4021.

Hall PF (1980). The roles of cytochromes P-450 in the synthesis and metabolism of steroids. In 3rd European meeting on cytochrome P-450, Saltsjöbaden, Sweden 1980; Biochemistry, biophysics and regulation of cytochrome P-450. Eds Gustafsson JÅ, Carlstedt-Duke J, Mode A, & Rafter J, pp 461-476. Amsterdam; Elsevier/north Holland biomedical press.

Hancock KW, & Level MJ (1978). Primidone/dexamethasone interaction. *Lancet* ii: 97-98.

Hanson JM, & Morley J (1985). Pharmacological aspects of glucocorticosteroids. In glucocorticosteroids, inflammation and bronchial hyperreactivity. Symposium at the 3rd congress of the European society of Pneumology, Basel, sept 19, 1984. Ed Hogg JC, Ellul-Micallef R, & Brattsand R, pp 11-20, Amsterdam: Excerpta Medica.

Haque N, Thrasher K, Werk EE Jr, Knowles HC Jr, & Sholiton LT (1972). Studies on dexamethasone metabolism in man: effect of diphenylhydantoin. *J Clin Endocr Metab* 34, 44-50.

Harter JG, Reddy WJ, & Thorn GW (1963). Studies on an intermittent corticosteroid dosage regimen. *N Engl J Med* 269, 591-596.

Hartiala J (1976). Steroid metabolism in adult lung. *Agents and Actions*, 6/4, 522-526.

Haynes RC, & Murad F (1980). Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogues; inhibitors of adrenocortical steroid biosynthesis. In *The pharmacological basis of therapeutics*, 6th ed. Eds Goodman Gilman A, Goodman LS, & Gilman A, pp 1466-1496. New York; Macmillan publishing Co, Inc.

Hench PS, Kendall EC, Slocumb CH, & Polley HF (1949). The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: Compound E) and of pituitary adrenocorticotrophic hormone on rheumatoid arthritis; preliminary report. *Staff Meet Mayo Clinic* 24, 181-197.

Hierholzer K, Lichtenstein I, Siebe H, Tsiakiras D, & Witt I (1982). Renal metabolism of corticosteroid hormones. *Klin Wochenschr*, 60, 1127-1135.

Higgins SJ, Baxter JD, & Rousseau GG (1979). Nuclear binding of glucocorticoid receptors. In *Glucocorticoid hormone action*. Monographs in endocrinology 12. Eds Baxter JD, & Rousseau GG, pp 136-160. Heidelberg: Springer-Verlag Berlin.

Hornke I, Cavagna F, Christ O, Fehlhaber H-W, Kellner H-M & Sandow J (1980). Pharmakokinetik und metabolismus von Desoxime-tason. *Arzneim-Forsch/Drug Res* 30, 47-54.

Hsia SL (1971). Steroid metabolism in human skin. In *Modern trends in dermatology*. Ed Borrie P, pp 69-88. London: Butterworth.

Johansson SÅ, Andersson KE, Brattsand R, Gruvstad E, & Hedner P (1982). Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. *Eur J Clin Pharmacol* 22, 523-529.

Johansson SÅ (1983). Evaluation of budesonide - a new glucocorticoid for local treatment of bronchial asthma. Thesis. University of Lund, Sweden.

Jori A, Di Salle E, & Santini V (1971). Daily rhythmic variation and liver drug metabolism in rats. *Biochem Pharm* 20, 2965-2969.

Jubiz W, Meikle AW, Levinson RA, Mizutani S, West CD, & Tyler FH (1970). Effect of diphenylhydantoin on the metabolism of dexamethasone. *N Engl J Med* 283, 11-14.

Jubiz W, & Meikle AW (1979). Alterations of glucocorticoid actions by other drugs and disease states. *Drugs* 18, 113-121.

Junge W (1977). Human microsomal carboxylesterase (EC 3.1.1.1). *Enzymes in health and Disease Inaug Scient Meet Int Soc Clin Enzymol*, London, pp 37-58.

Kaliner M (1985). Mechanisms of glucocorticosteroid action in bronchial asthma. *J Allergy Clin Immunol* 76, 320-329.

Kasuya Y, Althaus JR, Freeman JP, Mitchum RK, & Skelly JP (1984). Quantitative determination of dexamethasone in human plasma by stable isotope dilution mass spectrometry. *J Pharm Sci* 73, 446-451.

Kato R (1974). Sex-related differences in drug metabolism. *Drug Metab Rev* 3, 1-32.

Kawai S, Ichikawa Y, & Homma M (1985). Differences in metabolic properties among cortisol, prednisolone, and dexamethasone in liver and renal diseases: Accelerated metabolism of dexamethasone in renal failure. *J Clin Endocrin Metab* 60, 848-854.

Kershbaum A, Pappajohn DJ, Bellet S, Hirabayashi M, & Shafiiha H (1968). Effect of smoking and nicotine on adrenocortical secretion. *JAMA* 203, 275-278.

Kolanowski J, Corcelle-Cerf F, & Lammerant J (1981). Cortisol uptake, release and conversion into cortisone by the heart muscle in dogs. *J Steroid Biochem* 14, 773-781.

Kornel L, & Saito Z (1974). Studies on steroid conjugates - VIII: isolation and characterization of glucuronide-conjugated metabolites of cortisol in human urine. *J Steroid Biochem* 6, 1267-1284.

Kozower M, Veatch L, & Kaplan MM (1974). Decreased clearance of prednisolone, a factor in the development of corticosteroid side effects. *J Clin Endocrin Metab* 38, 407-413.

Kripalani KJ, Cohen AI, Weliky I & Schreiber EC (1975). Metabolism of triamcinolone acetonide-21-phosphate in dogs, monkeys, and rats. *J Pharm Sci* 64, 1351-1359.

Kripalani KJ, Zern El-Abdin A, Dean AV, & Cohen AI (1977). Bio-transformation of halcinonide in the dog. *The Pharmacologist* 19, 168.

Kuntzman R, Jacobson M, & Conney AH (1966). Effect of phenyl-butazone on cortisol metabolism in man. *Pharmacologist* 8, 195.

Kupfer D, & Partridge R (1970). 6 β -hydroxylation of triamcinolone acetone by a hepatic enzyme system. The effect of phenobarbital and 1-benzyl-2-thio-5,6-dihydrouracil. *Arch Biochem Biophys* 140, 23-28.

Kwaselow A, Mc Lean J, Busse W, Bush R, Reed C, Metzger W, Richerson H, Shulan D, Koshiver J, & Chaplin M (1985). A comparison of intranasal and oral flunisolide in the therapy of allergic rhinitis. Evidence for a topical effect. *Allergy* 40, 363-367.

Lambe R, Hudson S, O'Kelly D & Darragh A (1978). The metabolism of fluocortolone in the ram. *Ir J Med Sci* 147, 393-403.

Lanes S, Stevensson JS, Codias E, Hernandez A, Sielczak MW, & Abraham WM (1985). Effects of budesonide on late bronchial responses and the associated airway hyperresponsiveness in allergic sheep. In *Glucocorticoids, inflammation and bronchial hyperreactivity. Symposium at the 3rd congress of the European Society of Pneumology, Basel, sept 19, 1984.* Ed Hogg JC, Ellul-Micallef R, & Brattsand R, pp 38-50 Amsterdam: Excerpta Medica.

Langhoff E, Madsen S, Flachs H, Olgaard K, Ladefoged J, & Hvidberg EF (1985). Inhibition of prednisolone metabolism by cyclosporine in kidney-transplanted patients. *Transplantation* 39, 107-109.

Laurent H, Gerhards F, & Wiechert R (1975). New biologically active pregnan-21-oic acid esters. *J Steroid Biochem* 6, 185-192.

Lee HJ (1977). Acidic metabolite of prednisolone. *Experientia* 33, 253-254.

Lee HJ, & Soliman MRI (1982). Antiinflammatory steroids without pituitary-adrenal suppression. *Science* 215, 989-991.

Levitz M, Jansen V, & Dancis J (1978). The transfer and metabolism of corticosteroids in the perfused human placenta. *Am J Obstet Gynecol* 132, 363-366.

Linder CWR (1964). Adrenal suppression by aerosol steroid inhalation. *Arch Intern Med* 113, 655-666.

Lorenzetti OJ (1981). Topical steroid therapy. In *Pharmacological and biochemical properties of drug substances*, vol 3. Ed Goldberg ME pp 369-394. Washington: American Pharmaceutical Association.

Mannering GJ (1981). Hepatic cytochrome P-450 linked drug-metabolizing systems. In *concepts in drug metabolism. Part two.* Eds Jenner P, & Testa B, pp 53-166. New York: Marcel Dekker Inc.

- Martin LE, Tanner RJN, Clark TJH, & Cochrane GM (1973). Absorption and metabolism of orally administered beclomethasone dipropionate. *Clin Pharm Ther* 15, 267-275.
- Martinelli M, Ferrari P, Ripamonti A, Tuan G, Perazzi A & Assandri A (1979). Metabolism of deflazacort in the rat, dog and man. *Drug Metab Disp* 7, 335-339.
- McAllister WAC, Mitchell DM, & Collins JV (1981a). Prednisolone pharmacokinetics compared between night and day in asthmatic and normal subjects. *Br J Clin Pharmacol* 11, 303-304.
- McAllister WAC, Winfield CR, & Collins JU (1981b). Pharmacokinetics of prednisolone in normal and asthmatic subjects in relation to dose. *Eur J Clin Pharmacol* 20, 141-145.
- McCafferty J, Brophy TROR, Yelland JD, Cham BE, Bochner F, & Eadie MJ (1981). Intraoperative pharmacokinetics of dexamethasone. *Br J Clin Pharmacol* 12, 434-435.
- Meffin PJ, Wing LMH, Sallustio BC, & Brooks PM (1984a). Alterations in prednisolone disposition as a result of oral contraceptive use and dose. *Br J Clin Pharmacol* 17, 655-664.
- Meffin PJ, Brooks PM, & Sallustio BC (1984b). Alterations in prednisolone disposition as a result of time of administration, gender and dose. *Br J Clin Pharmacol* 17, 395-404.
- Melby JC (1977). Clinical Pharmacology of systemic corticosteroids. *Ann Rev Pharmacol Toxicol* 17, 511-527.
- Michels MI, Smith RE, & Heimlich EM (1967). Adrenal suppression and intranasally applied steroids. *Ann Allergy* 25, 569-574.
- Milsap RL, George DE, Szeffler SJ, Murray KA, Lebenthal E, & Jusko WJ (1983). Effect of Inflammatory bowel disease on absorption and disposition of prednisolone. *Dig Dis Sci* 28, 161-168.
- Milsap RL, Plaisance KI, & Jusko WJ (1984). Prednisolone disposition in obese men. *Clin Pharmacol Ther* 36, 824-831.
- Miyabo S, Hisada T, Kishida S, & Asato T (1976). Metabolism of synthetic corticosteroid esters in man. *Folia endocrinol jap* 52, 997-1007.
- Miyachi Y, Yotsumoto H, Kano T, Mizuchi A, Muto T, & Yanagibashi K (1979). Blood levels of synthetic glucocorticoids after administration by various routes. *J Endocr* 82, 149-157.
- Mizuchi A, Miyachi Y, Tamaki K, Kukila A (1976). Percutaneous absorption of betametasone 17-benzoate measured by radioimmunoassay. *J Invest Dermatol* 67, 279-282.
- Monder C, & Bradlow HL (1977). Carboxylic acid metabolites of steroids. General review. *J Steroid Biochem*, 8, 897-908.

Monder C, & Bradlow HL (1980). Corticoid acids: Explorations at the frontier of corticosteroid metabolism. *Rec Progr Horm Res* 36, 345-400.

Morris HG (1985). Mechanisms of glucocorticoid action in pulmonary disease. *Chest* 88, 1335-1415.

Morselli PL, Marc V, Garathini S, & Zaccala M (1970). Metabolism of exogenous cortisol in humans; I. Diurnal variations in plasma disappearance rate. *Biochem Pharmacol* 19, 1643-1647.

Mützel W (1977). Pharmakokinetik und Biotransformation von Fluo-cortin-bulytester beim Menschen. *Arzneim-Forsch/ Drug Res* 27, 2230-2233.

Mygind N (1982). Topical steroid treatment for allergic rhinitis and allied conditions. *Clin Otolaryngol* 7, 343-352.

Myers C, Lockridge O, & LaDu BN (1982). Hydrolysis of methylprednisolone acetate by human serum cholinesterase. *Drug Metab Disp* 10, 279-280.

Möllmann H, Rohdewald P, Schmidt EW, Salomon V, & Derendorf H (1985). Pharmacokinetics of triamcinolone acetonide and its phosphate ester. *Eur J Clin Pharmacol* 29, 85-89.

Nakano M, Nishiuchi M, Takeuchi M, & Yamada H (1981). Correlation between metabolism of betamethasone 17,21-dipropionate and adrenal hypertrophy in rat fetuses. *Steroids* 37, 511-525.

Narang PK, Wilder R, Chatterji DC, Yeager RL, & Gallelli JF (1983). Systemic bioavailability and pharmacokinetics of methylprednisolone in patients with rheumatoid arthritis following 'high-dose' pulse administration. *Biopharm & Drug Disp* 4, 233-248.

Newman SP (1985). Aerosol deposition considerations in inhalation therapy. *Chest* 88, suppl., 153S-160S.

Newman SP, Morén F, & Clarke SW (1986). Deposition patterns of aerosols from nasal pumps. Abstract. Eleventh Eur Rhinologic Society Congress.

Nicholas TH, & Lugg MA (1982). The physiological significance of 11 β -hydroxysteroid dehydrogenase in the rat lung. *J Steroid Biochem* 17, 113-118.

Nies AS, Shand DG, & Wilkinson GR (1976). Altered hepatic blood flow and drug disposition. *Clin Pharmacokinet* 1, 135-155.

Norman PS, Winkenwerder W, Murgatroyd G, & Parsons J (1966). Evidence for the local action of intranasal dexamethasone aerosols in the suppression of hay fever symptoms. *J Allergy* 38, 93-97.

Novey HS, & Beall G (1965). Aerosolized steroids and induced cushing's syndrome. *Arch Intern Med* 115, 602.

- Oishi T, Deguchi T, & Marumo H (1981). Metabolic fate of beclomethasone 17,21-dipropionate. *Oyo Yakori* 22, 717-727.
- Pakes GE, Brogden RN, Heel RC, Speight TM, & Avery GS (1980). Flunisolide: A review of its pharmacological properties and therapeutic efficacy in rhinitis. *Drugs* 19, 397-411.
- Pal SB (1978). 6-Hydroxylation of cortisol and urinary 6 β -hydroxycortisol. *Metabolism* 27, 1003-1011.
- Papen CV, Benker G, Hackenberg K, & Reinwein D (1982). Pharmakokinetik von Prednisolon bei Niereninsuffizienz. *Klin Wochenschr* 60, 681-686.
- Peets EA, Staub M, & Synchowitz S (1969). Plasma binding of betamethasone-3H, dexamethasone-3H, and cortisol-14C - a comparative study. *Biochem Pharmacol* 18, 1655-1663.
- Petereit LB, & Meikle AW (1977). Effectiveness of prednisolone during phenytoin therapy. *Clin Pharmacol Ther* 22, 912-916.
- Petersen MC, Nation RL, McBride WG, Ashley JJ, & Moore RG (1983). Pharmacokinetics of betamethasone in healthy adults after intravenous administration. *Eur J Clin Pharmacol* 25, 643-650.
- Peterson RE, Wyngaarden JB, Guerra SL, Brodie BB, & Bunim JJ (1955). The physiological disposition and metabolic fate of hydrocortisone in man. *J Clin Invest* 34, 1779-1794.
- Peterson RE (1960). Adrenocortical steroid metabolism and adrenal cortical function in liver disease. *J Clin Invest* 39, 320-331.
- Pickup ME, Lowe JR, Leatham PA, Rhind VM, Wright V, & Downie, WW (1977). Dose dependent pharmacokinetics of prednisolone. *Eur J Clin Pharmacol* 12, 213-219.
- Pipkorn U, Rundcrantz H, & Lindqvist N (1980). Budesonide - a new nasal steroid. *Rhinology* 18, 171-175.
- Pipkorn U (1982). Mechanisms of action and effects of topical glucocorticoids in pollen-induced allergic rhinitis. A clinical and experimental study with special reference to budesonide. Thesis. University of Gothenburg, Sweden.
- Quigley ME, & Yen SSC (1979). A mid-day surge in cortisol levels. *J Clin Endocrin Met* 49, 945-946.
- Radzialowski FM, & Bousquet WF (1967). Circadian rhythm in hepatic drug metabolism activity in the rat. *Life Science* 6, 2545-2548.
- Rafestin-Oblin ME, Michaud A, Claire M, Nakane H, & Corvol P (1977). Tritiated 9 α -fluorocortisol metabolism and binding in rat kidney. *Steroids* 30, 605-619.
- Rane A, Wilkinson GR, & Shand DG (1977). Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. *J Pharm Exp Ther* 200, 420-424.

- Reach G, Nakane H, Nakane Y, Auzan C, & Corvol P (1977). Cortisol metabolism and excretion in the isolated perfused rat kidney. *Steroids* 30, 605-619.
- Reichstein T, & Shoppee CW (1943). The hormones of the adrenal cortex. *Vitam Horm* 1, 346-413.
- Rice MJ, Tredger JM, Chakraborty J, & Parke D.V (1974). The metabolism of dexamethasone in the rat. *Biochem Soc Trans* 2, 107-109.
- Rocci ML, Szeffler SJ, Acara MA, & Jusko WJ (1981). Prednisolone metabolism and excretion in the isolated perfused rat kidney. *Drug Metab Disp* 9, 177-182.
- Ronca-Testoni S, & Rotondara L (1983a). Absorption and metabolism of cyclomethasone. *Drugs Exptl Clin Res* 9, 73-76.
- Ronca-Testoni S (1983b). Hydrolysis of cyclomethasone by the human lung. *Int J Clin Pharm Res* 3, 17-20.
- Rose J, Yurchak A, Meikle M, & Jusko W (1981a). Effect of smoking on prednisone, prednisolone and dexamethasone pharmacokinetics. *J Pharmacokin Biopharm* 9, 1-14.
- Rose JQ, Yurchak AM, & Jusko WJ (1981b). Dose dependent pharmacokinetics of prednisone and prednisolone in man. *J Pharmacokin Biopharm* 9, 389-417.
- Rousseau GG, Baxter JD, & Tomkins GM (1972). Glucocorticoid receptors; relations between steroid binding and biological effects. *J Molec Biol* 67, 99-115.
- Rowland M, & Scheiner LB (1985). Lecture 4. Physiological models - distribution and elimination. In Workshop in advanced methods in pharmacokinetics. University of Manchester and University of California, San Fransisco.
- Ryrfeldt Å, Tönnesson M, Nilsson E, & Wikby A (1979). Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography. *J Steroid Biochem* 10, 317-324.
- Ryrfeldt Å, Andersson P, Edsbäcker S, Tönnesson M, Davies D, & Pauwels R (1982). Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis.* 63, Suppl 122, 86-95.
- Schalm SW, Summerskill WHJ, & Go VLW (1977). Prednisone for chronic active liver disease: Pharmacokinetics, including conversion to prednisolone. *Gastroenterology* 72, 910-913.
- Schrievers H (1967). Factors regulating the metabolism of steroids. *Vitam Horm* 25, 271-314.

Schuetz EG, Wrighton SA, Barwick JL, & Guzelian PS (1984). Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16 α -carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver in vivo. *J Biol Chem*, 1999-2006.

Shapiro GG, & Christie DL (1983). Gastroesophageal reflux and asthma. *Clin Rev Allerg* 1, 39-56.

Siegel SC (1985). Overview of corticosteroid therapy. *J Allergy Clin Immunol* 66, 447-451.

Skrabalak DS, & Maylin GA (1982). Dexamethasone metabolism in the horse. *Steroids* 39, 233-244.

Somogyi A, & Gugler R (1982). Drug interactions with cimetidine. *Clin Pharmacokin* 7, 23-41.

Stjernholm MR, & Katz FH (1975). Effects of diphenylhydantoin, phenobarbital, and diazepam on the metabolism of methylprednisolone and of its sodium succinate. *J Clin Endocrinol Metab* 41, 887-893.

Swartz SL, & Dluhy RG (1978). Corticosteroids: Clinical pharmacology and therapeutic use. *Drugs* 16, 238-255.

Szeffler SJ, Rose JQ, Ellis EF, Spector SL, Green AW, & Jusko WJ (1980). The effect of troleandomycin on methylprednisolone elimination. *J Allergy Clin Immunol* 66, 447-451.

Szeffler SJ, Ellis EF, Brenner M, Rose JQ, Spector SL, Yurchak AM, Andrews F, & Jusko WJ (1982). Steroid-specific and anticonvulsant interaction aspects of troleandomycin-steroid therapy. *J Allergy Clin Immunol* 69, 455-460.

Särnstrand B (1985). Effects of glucocorticoids on proteoglycan and hyaluronic acid metabolism in fibrous connective tissue. Thesis. University of Lund, Sweden.

Tan K, Kumakura M, Kobari T, Watanabe S, Kobayashi H, & Namekawa H (1976). *Pharmacometrics (Sendai)* 12, 351-362.

Tanner A, Bochner F, Caffin J, Halliday J, & Powell L (1979). Dose-dependent prednisolone kinetics. *Clin Pharmacol Ther* 25, 571-578.

Teitelbaum PJ, Chu NI, Cho D, Tökés L, Patterson JW, Wagner PJ & Chaplin MD (1981). Mechanism for the oxidative defluorination of flunisolide. *J Pharm Exp Ther* 218, 16-22.

Thalén A, & Brattsand R (1979). Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with a high local activity. *Arzneim Forsch/Drug Res* 29, 1687-1690.

Toothaker RD, Craig WA, & Welling PG (1982). Effect of dose size on the pharmacokinetics of oral hydrocortisone suspension. *J Pharm Sci* 71, 1182-1185.

Toothaker RD, & Welling PG (1983). Effect of dose size on the pharmacokinetics of intravenous hydrocortisone during endogenous hydrocortisone suppression. *J Pharmacokin Biopharm* 10, 147-156.

Tredger JM, Chakraborty J, & Parke DV (1973). a comparative study of the excretion of corticosterone and betamethasone in the rat. *Biochem Soc Trans* 1, 998-999.

Tse CST, & Bernstein IL (1984). Corticosteroid aerosols in the treatment of asthma. *Pharmacotherapy* 4, 334-342.

Tsuei SE, Moore RG, Ashley JJ, & McBride WG (1979). Disposition of synthetic glucocorticoids. I. Pharmacokinetics of dexamethasone in healthy adults. *J Pharmacokin Biopharm* 7, 249-265.

Tsuei SE, Petersen MC, Ashley JJ, McBride WG, & Moore RG (1980). Disposition of synthetic glucocorticoids; II. Dexamethasone in parturient women. *Clin Pharmacol Ther* 28, 88-98.

Täuber U, & Toda T (1976). Biotransformation von Diflucortolon-valerianat in der Haut von Ratte, Meerschweinchen und Mensch. *Arzneim Forsch/Drug Res* 26, 1484-1498.

Täuber U, Haack D, Nieuweboer B, Kloss G, Vecsei P, & Wendt H (1984). The pharmacokinetics of flucortolone and prednisolone after intravenous and oral administration. *Int J Clin Pharmacol Ther Toxicol* 22, 48-55.

Tökés L, Cho D, Maddox ML, Chaplin MD, & Chu NI (1981). Isolation and identification of an oxidatively defluorinated metabolite of flunisolide in man. *Drug Metab Disp* 9, 485-486.

Uribe M, & Go VLW (1978). Research review. Corticosteroid pharmacokinetics in liver disease. *Clinical Pharmacokinetics* 4, 233-240.

Uribe M, Summerskill WHJ, & Go VLW (1977). Why hyperbilirubinemia and hypoalbuminemia predispose to steroid side effects during treatment of chronic active liver disease (abstract). *Gastroenterology* 72, 1143.

Vermeulen A, & Caspi E (1958). The metabolism of prednisolone by homogenates of rat liver. *J Biol Chem* 233, 54-56.

Vermeulen A (1959). The metabolism of 4-¹⁴C prednisolone. *J Endocrin* 18, 278-291.

Walker CH (1978). Species differences in microsomal activity and their relationship to biological half-lives. *Drug Metab Rev* 7, 295-323.

Wilkinson GR, & Shand DG (1975). A physiological approach to hepatic drug clearance. Clin Pharmacol Ther 18, 377-390.

Williams FM (1985). Clinical significance of esterases in man. Clinical Pharmacokinetics 10, 392-403.

Woodhouse KW, Mutch E Williams FM, Rawlins MP, & James OFW (1984). The effect of age on pathways of drug metabolism in human liver. Age and ageing 13, 328-334.

Zumoff B, Bradlow L, Gallagher TF, & Hellman L (1967). Cortisol metabolism in cirrhosis. J Clin Invest 46, 1735-1743.

Øje S, & Tozer TN (1979). Effect of altered plasma protein binding on apparent volume of distribution. J Pharm Sci 68, 1203-1205.

Öst L (1984). Effects of cyclosporine on prednisolone metabolism. Lancet 1, 451.

METABOLIC PATHWAYS OF THE TOPICAL GLUCOCORTICOID BUDESONIDE IN MAN

S. EDSBÄCKER, S. JÖNSSON, C. LINDBERG, Å. RYRFELDT, AND A. THALÉN

Research and Development Department, AB Draco (subsidiary of AB Astra) (S. E., S. J., C. L., Å. R., A. T.); and Department of Clinical Pharmacology, University Hospital of Lund (S. E.)

(Received June 1, 1983; accepted July 12, 1983)

ABSTRACT:

The metabolic pathways of budesonide[(22R)-16 α ,17 α -butyridenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione] in human liver 9000g supernatant fraction were studied. A comparison was made between the *in vitro* metabolite pattern and the metabolite pattern in plasma obtained after iv administration of tritiated budesonide to man. No qualitative difference could be found, which indicates that the *in vitro* model is useful to predict results *in vivo*. The two major metabolites formed *in vitro* were identified by HPLC and mass spectrometry as 6 β -hydroxybudesonide and 16 α -

hydroxyprednisolone. Loss of the acetal group was not observed when desonide (11 β ,21-dihydroxy-16 α ,17 α -isopropylidenedioxy-pregna-1,4-diene-3,20-dione) was incubated with human liver 9000g supernatant fraction. Neither could 16 α -hydroxyprednisolone be detected after incubation of the (22S)-epimer of budesonide with the same medium. The cleavage of the acetal moiety is therefore suggested to be the result of a substrate-selective metabolic pathway.

Budesonide [(22R)-16 α ,17 α -butyridenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione, fig. 1] is a potent nonhalogenated glucocorticoid, effective in the local treatment of inflammatory and allergic disorders, such as psoriasis (1), asthma (2), and rhinitis (3). The drug is a 1:1 mixture of two epimers with (22R) and (22S) configuration (4), which both have the same qualitative pharmacological effects, the epimer (22R) being 2–3 times more potent than epimer (22S) (5). Since glucocorticoid action at present is believed to be mediated via identical receptors throughout the organism (6), receptor differentiation should not explain the favorable ratio between topical antiinflammatory and systemic glucocorticoid activity observed for budesonide (5, 7, 8). Anderson *et al.* (9) have shown that budesonide is rapidly metabolized in liver preparations of rat, hairless mouse, and man. These results, together with a demonstrated increased systemic activity of budesonide in SKF 525-A-pretreated rats (8), support the hypothesis that the low systemic effects of the drug are due to rapid biotransformation into metabolites with low activity.

The aim of the present investigation was to isolate and identify metabolites formed *in vitro* after incubation of budesonide with human liver 9000g supernatant fraction. To investigate the significance of the results obtained *in vitro*, comparison was made with the metabolite pattern in man after iv administration of budesonide.

Materials and Methods

Chemicals. Budesonide was prepared from 16 α -hydroxyprednisolone and *n*-butyraldehyde in the presence of an acid catalyst (10). Separation of the budesonide (22R)- and (22S)-epimers was performed as previously described (11). Deuterated budesonide [$^2\text{H}_8$]budesonide; (22R,S)-(22,23,23,24,24,25,25,25- H_8)-16 α ,17 α -butyridenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione) was synthesized similar to nonlabeled budesonide using *n*-($^2\text{H}_8$)butyraldehyde (12).

This work was presented in part at the Stowe School Symposium on Drug Metabolism, Buckingham, England, April 4–7, 1982.

Send reprint requests to: Staffan Edsbacker, M.S., Pharmacokinetic Laboratory, AB Draco, Box 1707, S-221 01 Lund, Sweden.

Tritiated budesonide ([^3H]budesonide; (22R,S)-16 α ,17 α -butyridenedioxy-11 β ,21-dihydroxy[1,2- ^3H]pregna-1,4-diene-3,20-dione) with a specific activity of 76.6 mCi/mg (2.83 GBq/mg) was obtained from the Radiochemical Centre, Amersham, U.K. The compound was purified according to a method of Andersson *et al.* (9). Subsequent HPLC analysis showed a radiochemical purity of >95%.

16 α -Hydroxyprednisolone (11 β ,16 α ,17 α ,21-tetrahydroxypregna-1,4-diene-3,20-dione) and desonide (11 β ,21-dihydroxy-16 α ,17 α -isopropylidenedioxy-pregna-1,4-diene-3,20-dione) were supplied by Lark S.p.a., Milan, Italy. The synthesis of 6 α - and 6 β -hydroxybudesonide will be reported elsewhere.

All organic solvents were of spectroscopic grade and glass-distilled before use. Water filtered through a Millipore Super Q 4 module system was used. D-Glucose-6-phosphate, NADP $^+$, and glucose-6-phosphate dehydrogenase were obtained from Sigma, St. Louis, MO.

Liver Preparation. Specimens of three human livers were obtained from the liver bank at Huddinge Hospital, Sweden by the courtesy of Dr. C. von Bahr. The livers were taken from one woman (age 53) and two men (ages 18 and 24) shortly after circulatory arrest, and immediately frozen at -196°C . They were then stored at -80°C before use. Storage under these conditions preserves most enzyme activities for at least 6 months (13). The livers were separately homogenized for 15 sec in 2.5 volumes of 0.2 M sucrose/0.1 M phosphate buffer, pH 7.4, with a Polytron PT20 homogenizer (Kinematica, Luzern, Switzerland) and by 10 strokes with a Teflon pestle operating at 3000 rpm. The homogenate was then centrifuged at 9000g, and the protein concentration of the supernatant fraction was estimated with bovine serum albumin as standard (14). The preparation was carried out at 4°C . The supernatant fraction was stored frozen at -80°C before use. No decrease in enzyme activity was noticed during the storage.

Incubation Medium. The 9000g supernatant fraction from the liver homogenate was diluted with homogenizing buffer to a protein concentration of 10 mg/ml, and incubated in the presence of MgCl_2 (10 mM), glucose-6-phosphate (75 mM), NADP $^+$ (3.0 mM), and glucose-6-phosphate dehydrogenase (4.3 units/ml). The incubation experiment for comparison with the metabolite pattern in human plasma was performed in a medium containing 1 mg/ml protein, 10 mM MgCl_2 , 7.5 mM glucose-6-phosphate, 0.3 mM NADP $^+$, and 0.4 unit/ml glucose-6-phosphate dehydrogenase (9). All incubations were performed at 37°C under a carbon atmosphere (95% O_2 + 5% CO_2) with gentle shaking.

HPLC. The high performance liquid chromatograph consisted of a model 660 solvent programmer, two M6000 A pumps, and a M440 UV-

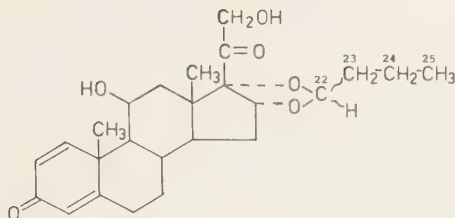


Fig. 1. Structural formula of budesonide.

detector ($\lambda = 254$ nm) from Waters Associates, Milford, MA. A model CV-6-UHPA-N60 (Valco Instruments Co, Houston, TX.) injector equipped with a 250- μ l loop and a model B100 fraction collector (Gilson, Villiers le Bel, France) were also used. A 45-min solvent program was run using a linear or slightly concave gradient from 25 to 60% (v/v) ethanol in water. Analysis was performed on a 5 $\times$ 50 mm Corasil C₁₈ precolumn (Waters Associates) connected to a 5 $\times$ 200 mm analytical column, slurry packed with Nucleosil C₁₈, 5- μ m particles (Macherey-Nagel, Düren, West Germany). One-min fractions were collected for radioactivity measurements.

Radioactivity Measurements. Radioactivity was measured by the liquid scintillation technique. The measurements were performed in Instagel using a Tricarb 460 CD (Packard Instruments, Downers Grove, IL) liquid scintillation counter equipped with external standard for determination of counting efficiency.

Incubation and Extraction of Metabolites for Mass Spectrometric Identification. A mixture of budesonide/(³H)₆budesonide/(³H)budesonide, 5000:5000:1 (w/w/w) was added to 20 ml of the incubation medium to a total steroid concentration of 50 μ M. Three hours after the start of incubation, the medium was extracted twice with 15 ml of ethyl acetate. The organic phase, containing 91% of added radioactivity, was evaporated to dryness and the residue dissolved in 100 μ l of 30% (v/v) ethanol in water. The sample was run on the HPLC system described above, and appropriate fractions were diluted with 1 ml of water and further purified by extraction with ethyl acetate. The organic phase was evaporated and the residue dissolved in 100 μ l of 10% (v/v) ethanol in heptane. The samples, containing different budesonide metabolites, were chromatographed on a 5 $\times$ 200 mm Nucleosil NH₂ column, 10- μ m particles, using isocratic systems with 10% (compound V), 15% (compound III), and 20% (compound I) (v/v) ethanol in heptane as mobile phase. Fractions of 1 ml were collected, and those containing radioactivity were transferred into 5-ml vials and evaporated at 50°C under nitrogen.

Mass Spectrometry. Each sample was dissolved in 20–30 μ l of ethanol and applied with a microliter syringe to the end of a small copper rod (15 $\times$ 2 mm), from which the solvent was evaporated at 70°C. The copper rod was placed in the sample holder of a direct introduction probe, and introduced into the ion source of a Finnigan 4000 mass spectrometer (Finnigan MAT, San Jose, CA), interfaced to a model 6100 data system. Chemical ionization mass spectra were obtained using methane as the reagent gas at an ion source pressure of 0.4 torr. The temperature of the ion source was 250°C. Spectra were recorded repetitively while increasing the probe temperature up to 300°C at a rate of 95°C/min.

In Vivo Study. Plasma was obtained from six human subjects after iv injection of 500 μ g (100 μ Ci) of [³H]budesonide. The subjects were informed concerning the nature of the study and gave their written consent.¹ For comparison, [³H]budesonide was incubated with human liver 9000g supernatant fraction (9) for 1 h. The samples were extracted with ethyl acetate and the organic phases were evaporated at 50°C under nitrogen. The residues were dissolved in 200 μ l of 25% (v/v) ethanol in water and injected on the HPLC system described above.

Extraction on Disposable C₁₈ Cartridges. Extraction on disposable Sep-Pak C₁₈ cartridges (Waters Associates) was employed during the end of

this study. The procedure was more convenient than the ethyl acetate extraction procedure, and it resulted in cleaner extracts.

The cartridge was conditioned with 5 ml of ethanol followed by 5 ml of water. After application of the sample, the cartridge was washed with 10 ml of water and 10 ml of 10% (v/v) ethanol in water. The sample was eluted with 4 ml of 60% (v/v) ethanol in water, diluted with 20 ml of water, and applied to another conditioned Sep-Pak cartridge. The sample was finally eluted with 4 ml of ethanol into a borosilicate test tube, and evaporated at 50°C under a gentle stream of nitrogen. The residue was dissolved in 120 μ l of 25% (v/v) ethanol in water, and 40 μ l were injected on the HPLC system described above.

The recovery of [³H]budesonide through the whole extraction procedure was $93.2 \pm 3.4\%$ (mean $\pm$ SD, $N = 10$). During incubation of [³H]budesonide, at an initial concentration of 10 μ M, with human liver 9000g supernatant fraction, the recovery of radioactivity decreased from 90–95% at 0 min to 80–85% at 60 min incubation time. This indicates that metabolites are formed which have a lower affinity for the C₁₈ cartridge than budesonide itself.

In Vitro Biotransformation of the Budesonide Epimers and Desonide. Desonide, epimer (22R), or epimer (22S) of budesonide were added to 13 ml of the incubation medium to a concentration of 10 μ M. Aliquots (2.0 ml) were taken from each incubation flask at 0, 6, 15, 30, and 45 min after start of incubation. The samples were immediately frozen on dry ice (–60°C) and stored at –20°C until extraction. Two ml of [³H]budesonide solution (15 nM) were added to the frozen samples as a standard to allow estimation of recovery. After vigorous mixing, 3.5 ml were applied to a C₁₈ cartridge, and the extract was analyzed by HPLC.

The UV peak heights of the parent compound (desonide, epimer (22S), or epimer (22R) of budesonide) and 16 α -hydroxyprednisolone were measured, and divided by the amount of radioactivity eluted during the total chromatogram. This calculation compensated for nonquantitative recovery and variations in the volume injected on the HPLC column.

Results

The metabolite pattern observed after 60-min incubation of [³H]budesonide with human liver 9000g supernatant fraction was compared with that which appeared after iv injection of [³H]budesonide into man. Only minor differences could be found in the plasma metabolite pattern from the six subjects. Fig. 2 shows the radiochemical elution profiles of extracts obtained from the *in vitro* incubation and one of the subjects. Five radioactive compounds (I–V) could be separated, each peak in the plasma sample chromatogram having its counterpart in the *in vitro* sample chromatogram.

When 500 μ M SKF 525-A was added to the incubation medium, or when the NADPH-generating system was omitted, peak V increased and peaks I–IV all decreased in the chromatogram. When the 9000g supernatant fraction was heated to 80°C for 5 min prior to incubation, or when it was replaced with 100,000g supernatant fraction, only compound V could be detected. This compound coeluted with authentic budesonide. The results indicate that the formation of compounds I–IV are catalyzed by cytochrome P-450.

Fig. 3 shows the HPLC separation of an ethyl acetate extract, obtained after incubation of a mixture of budesonide, (³H)₆budesonide and [³H]budesonide with human liver 9000g supernatant fraction for 3 hr. Each of the shaded fractions (fractions 37 and 38 were pooled), corresponding to compounds I, III, and V, were purified by straight phase HPLC and analyzed by chemical ionization mass spectrometry.

The HPLC retention time of compound V corresponded to that of authentic budesonide (table 1), and as can be seen from fig. 3, chromatographic resolution of the two epimers (compound V, epimers 1 and 2) was obtained. A slight separation of deuterated budesonide from the unlabeled material is also evident. Fig. 4

¹ Å Rylfjeldt, S. Edsbacker, and R. Pauwels, manuscript in preparation

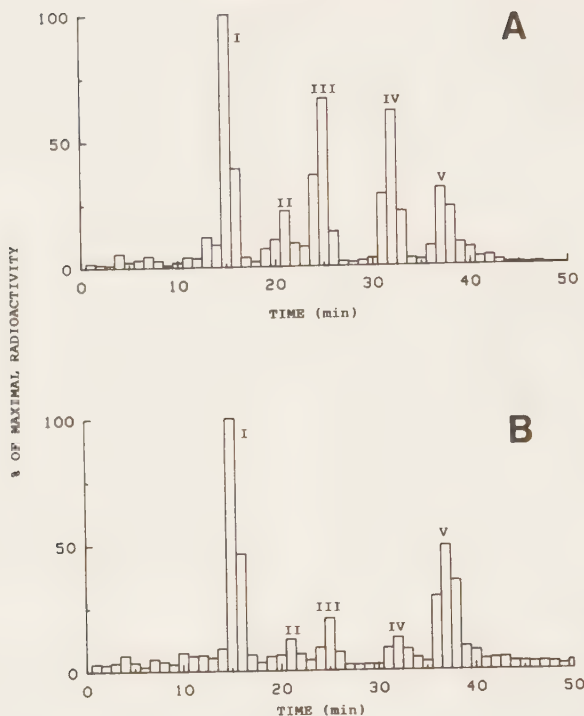


FIG. 2. Radiochemical elution profiles of two ethyl acetate extracts containing budesonide metabolites.

A, incubation of [^3H]budesonide with human liver 9000g supernatant fraction for 1 h. B, human plasma sample taken 30 min after iv injection of [^3H]budesonide. The separation was performed by HPLC as described under *Materials and Methods*. The peaks were designated compounds I-V, according to the order in which they eluted.

shows the mass spectra of compound V and authentic budesonide. If the two peaks at m/z 439 and 421, which are due to deuterium-labeled budesonide, are ignored, the two spectra are very similar.

Fig. 5 shows the mass spectrum of compound III, isolated from fraction 27 (*cf.* fig. 3). The isotope clusters at m/z 447/455, 429/437, and 411/419 show the expected doublets 8 atomic mass units apart, and indicate that the acetal group is intact. The peak at m/z 447, together with the deuterium isotope peak at m/z 455, indicates that the molecular weight is 16 atomic mass units higher than budesonide, and suggests that this compound is a hydroxylated metabolite of budesonide. Authentic 6 β -hydroxybudesonide showed a similar mass spectrum (fig. 5), and the HPLC retention time of one of its epimers (epimer 2) corresponded to that of compound III isolated from fraction 27 (table 1). The retention time of epimer 1 of 6 β -hydroxybudesonide corresponded to that of another, smaller peak in the chromatogram (*cf.* fig. 3 and table 1). A slight separation of deuterated from unlabeled material can be seen also with compound III. The retention time of the authentic 6 α -hydroxybudesonide epimers differed considerably from that of the compound III epimers (table 1).

Fig. 6 shows the mass spectrum of compound I. The absence of isotope clusters suggests that the acetal group, containing the deuterium atoms, has been lost. Cleavage of the 16 α ,17 α -acetal linkage would lead to the formation of 16 α -hydroxyprednisolone, and the small peak at m/z 377 corresponds to the MH^+ ion of this compound (molecular weight = 376). Comparison with authentic material revealed similar mass spectra (fig. 6) and corresponding HPLC characteristics (table 1), which identified compound I as 16 α -hydroxyprednisolone.

In order to evaluate the substrate selectivity of the enzymatic cleavage of the 16 α ,17 α -acetal linkage, the *in vitro* liver biotransformation of desonide and the (22*R*)- and (22*S*)-epimers of budesonide were separately followed, as well as the formation of 16 α -hydroxyprednisolone. The Sep-Pak extraction procedure, used in this experiment, gave a very pure sample, making quantification of the UV peaks possible. A monoexponential decline of the concentration of the parent compounds (correlation coefficients better than 0.96) suggests that the degradation follows first order kinetics. The t_1 was calculated to be 15, 27, and 84 min for the budesonide epimer (22*R*), epimer (22*S*), and desonide, re-

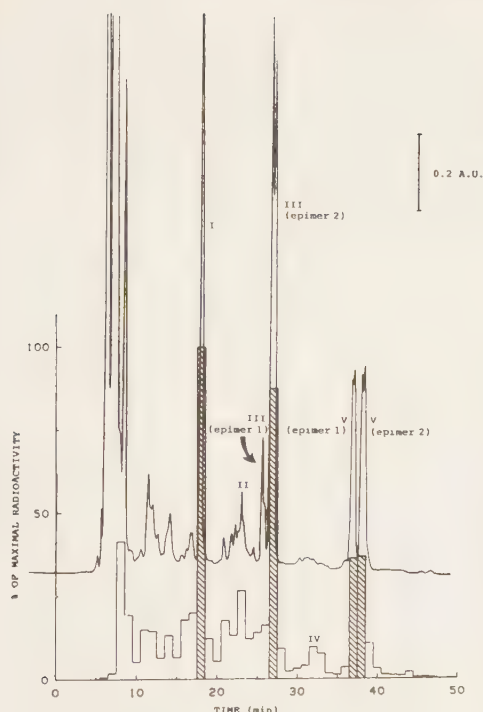


FIG. 3. HPLC analysis of an ethyl acetate extract of human liver 9000g supernatant fraction incubated with a mixture of budesonide, (^3H)budesonide, and (^3H)budesonide for 3 h.

The chromatographic conditions are described under *Materials and Methods*. The radiochemical elution profile and the UV recording are shown. The shaded fractions, belonging to compounds I, III, and V, were further processed for mass spectrometric identification.

spectively. The formation of 16 α -hydroxyprednisolone could be detected only after incubation of the (22R)-epimer of budesonide.

Discussion

Budesonide is a highly potent glucocorticoid, and very low plasma concentrations are found after administration of therapeutic doses to man (15). The possibility of direct isolation and identification of plasma metabolites is therefore limited. *In vitro* studies with liver preparations have been used extensively as a means of screening drug metabolism (9, 16–18). More seldom, attempts have been made to establish whether the observed results have any relevance to the *in vivo* situation. It has been shown that liver biotransformation is important in the inactivation of glucocorticoids, but qualitative and quantitative species differences exist (19–21). An *in vitro* system based on human liver should therefore be the most useful to predict metabolism in the clinical situation. The metabolite pattern obtained after incubation of (^3H)budesonide with human liver 9000g supernatant fraction conforms quali-

TABLE I

The retention time, relative to epimer (22S) of budesonide, of synthetic reference compounds, and compounds I, III, and V isolated from human liver 9000g supernatant fraction

The chromatographic conditions are described under *Materials and Methods*. Values are the mean of two chromatographic runs.

Compound*	Relative Retention Time
16 α -Hydroxyprednisolone	0.45
Compound I	0.45
6 α -Hydroxybudesonide	
epimer 1	0.57
epimer 2	0.59
6 β -Hydroxybudesonide	
epimer 1	0.68
epimer 2	0.71
Compound III	
epimer 1	0.67
epimer 2	0.70
Budesonide	
epimer (22R)	0.97
epimer (22S)	1.00
Compound V	
epimer 1	0.95
epimer 2	1.00

* Only the configuration of budesonide epimers are characterized.

tatively with that obtained after *iv* administration of the tritiated drug (fig. 2). The metabolites isolated and identified in this *in vitro* study are therefore suggested to be present also in human plasma.

Hydroxylation of the 6 β -position is a common pathway in the metabolism of glucocorticoids (22–24). Loss of the acetal group, however, has never been conclusively shown to be a metabolic pathway for glucocorticoid 16 α ,17 α -acetals. The formation of triamcinolone was suggested after *im* injection of triamcinolone acetonide to five patients with advanced breast cancer (25), but this finding was not confirmed in later studies. On the contrary, less than 3% of the ^{14}C label from the acetonide portion could be recovered as $^{14}\text{CO}_2$ after *iv* and *im* injection of triamcinolone acetonide 21-phosphate to rats, indicating no or minimal degradation of the acetonide moiety (20). After *iv* and *po* administration of [^{14}C]flunisolide (labeled in the acetonide position) to several animal species, 80–90% of the radioactivity was recovered in the excreta. The result was taken as an indication of no or minor loss of the acetonide group of flunisolide (21). Our study not only confirms the stability of the traditional symmetrical 16 α ,17 α -acetonide moiety for metabolic attack, but also show the metabolic pathway to be substrate selective for the (22R)-epimer of budesonide. Evidently, the asymmetry in the acetal group of budesonide, with only one alkyl substituent attached to the 22-carbon, makes this carbon vulnerable for biotransformation. Possibly, the absence of steric hindrance on the β -side of the dioxolane ring makes an attack by oxygen on C-22 possible in the cytochrome P-450-catalyzed oxidation. The oxidized intermediate is probably unstable and will immediately degrade into 16 α -hydroxyprednisolone. The substrate-selective nature of the reaction should, at least partly, explain the different *in vitro* biotransformation rates of the budesonide epimers.

The glucocorticoid activity of the identified metabolites is greatly reduced as judged from topical antiinflammatory and thymus involution tests as well as from glucocorticoid receptor affinity studies. Thus, the relative binding affinities of 6 β -hydroxybudesonide and 16 α -hydroxyprednisolone are only 0.008 and 0.004, respectively, of that reported for budesonide.²

Preliminary data on isolated compound IV have indicated a strongly reduced specific absorptivity at 200–300 nm, indicating

² E. Dahlberg, A. Thälén, R. Brattsand, J. Å. Gustafsson, U. Johansson, K. Roempke, and T. Saartok, manuscript in preparation

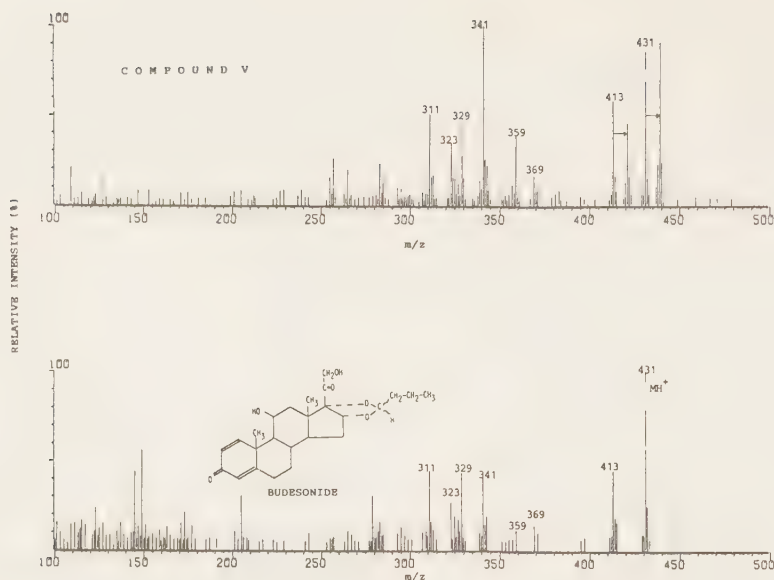


FIG. 4. Chemical ionization (methane) mass spectra of compound V and authentic budesonide.

Compound V was isolated from human liver 9000g supernatant fraction after incubation with a mixture of budesonide, (²H₈)budesonide, and [³H]budesonide. The arrows indicate deuterium-containing ions.

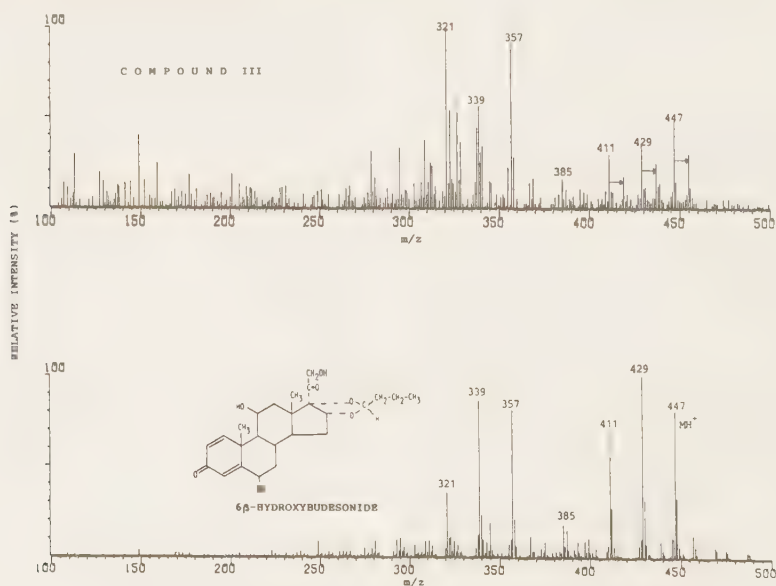


FIG. 5. Chemical ionization (methane) mass spectra of compound III and authentic 6β-hydroxybudesonide.

Compound III was isolated from human liver 9000g supernatant fraction after incubation with a mixture of budesonide, (²H₈)budesonide, and [³H]budesonide. The arrows indicate deuterium-containing ions.

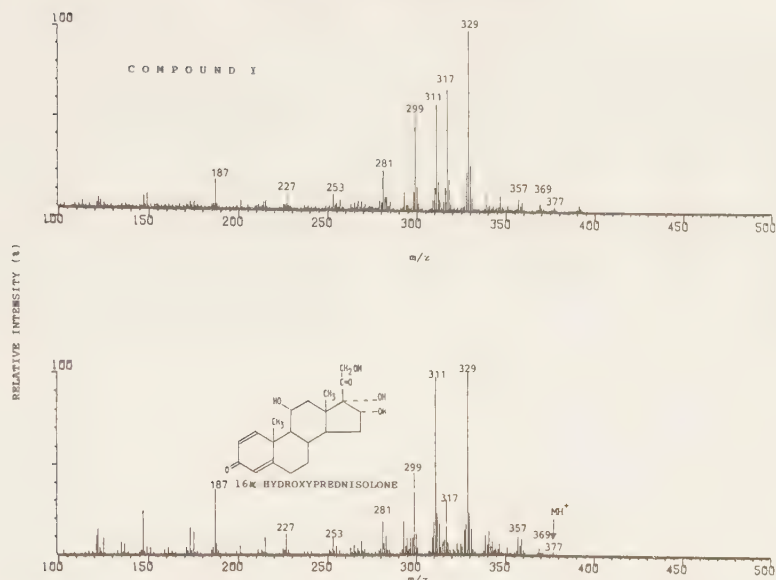


FIG. 6. Chemical ionization (methane) mass spectra of compound I and authentic 16 α -hydroxyprednisolone.

Compound I was isolated from human liver 9000g supernatant fraction after incubation with a mixture of budesonide, ($^2\text{H}_8$)budesonide, and [^3H]budesonide.

a biotransformation of the UV-absorbing 1,4-diene-3-one moiety of the drug. Work is in progress to isolate compounds II and IV in sufficient quantities to allow identification.

In conclusion, the two major metabolites of budesonide have been isolated and identified after incubation with human liver 9000g supernatant fraction. Our findings suggest that these metabolites are present also in human plasma after iv administration of the drug. Both metabolic pathways lead to a pronounced loss of glucocorticoid activity. The pathway producing 16 α -hydroxyprednisolone is suggested to be substrate selective for the (22R)-epimer of budesonide.

References

1. A. Hejzer, G. Hesser, P. Holm, and L. Salde: Comparison between two non-halogenated glucocorticoid ointments in psoriasis. *J. Int. Med. Res.* **9**, 239-247 (1981).
2. R. Ellul-Micallef, E. Hansson, and S. Å. Johansson: Budesonide: a new corticosteroid aerosol in bronchial asthma. *Eur. J. Respir. Dis.* **61**, 167-173 (1980).
3. U. Pipkorn, H. Rundcrantz, and N. Lindqvist: Budesonide—a new nasal steroid. *Rhinology* **18**, 171-175 (1980).
4. J. Albertsson, Å. Oskarsson, and L. Svensson: X-ray study of budesonide: molecular structures and solid solutions of the (22S) and (22R) epimers of 11- β ,21-dihydroxy-16- α ,17- α -propylmethylene-dioxy-1,4-pregnadiene-3,20-dione. *Acta Cryst.* **B34**, 3027-3036 (1978).
5. R. Brattsand, A. Thälén, K. Roempke, L. Källström, and E. Gruvstad: Influence of 16- α ,17- α -acetal substitution and steroid nucleus fluorination on the topical to systemic activity of glucocorticoids. *J. Steroid Biochem.* **16**, 779-786 (1982).
6. D. Feldman, J. Funder, and D. Loose: Is the glucocorticoid receptor identical in various target organs? *J. Steroid Biochem.* **9**, 141-145 (1978).
7. S. Å. Johansson, K. E. Andersson, R. Brattsand, E. Gruvstad, and P. Hedner: Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. *Eur. J. Clin. Pharmacol.* **22**, 523-529 (1982).
8. R. Brattsand, A. Thälén, K. Roempke, L. Källström, and E. Gruvstad: Development of new glucocorticosteroids with a very high ratio between topical and systemic activities. *Eur. J. Respir. Dis.* **63**, Suppl. 122, 62-73 (1982).
9. P. Andersson, S. Edsbacker, Å. Ryrfeldt, and C. von Bahr: *In vitro* biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J. Steroid Biochem.* **16**, 787-795 (1982).
10. A. Thälén and R. Brattsand: Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with high local activity. *Arzneim. Forsch. Drug Res.* **29**, 1687-1690 (1979).
11. A. Thälén and B. Nylander: Epimers of budesonide and related corticosteroids. I. Preparative resolution by chromatography on Sephadex LH-20. *Acta Pharm. Suec.* **19**, 247-266 (1982).
12. A. Thälén: Epimers of budesonide and related corticosteroids. II. Structure elucidation by mass spectrometry. *Acta Pharm. Suec.* **19**, 327-354 (1982).
13. C. von Bahr, C. G. Groth, H. Jansson, G. Lundgren, M. Lind, and H. Glaumann: Drug metabolism in human liver *in vitro*: establishment of a human liver bank. *Clin. Pharmacol. Ther.* **27**, 711-725 (1980).
14. O. H. Lowry, N. J. Rosebrough, A. H. Farr, and R. J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275 (1951).

15. Å Ryrfeldt, P. Andersson, S. Edsbäcker, M. Tönnesson, D. Davies, and R. Pauwels: Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur. J. Respir. Dis.* **63**, Suppl. 122, 86-95 (1982).
16. P. J. Teitelbaum, N. I. Chu, D. Cho, L. Tokés, J. W. Patterson, P. J. Wagner, and M. D. Chaplin: Mechanism for the oxidative defluorination of flunisolide. *J. Pharmacol. Exp. Ther.* **218**, 16-22 (1981).
17. D. Kupfer and R. Partridge: 6 β -Hydroxylation of triamcinolone acetate by a hepatic enzyme system. The effect of phenobarbital and 1-benzyl-2-thio-5,6-dihydrouracil. *Arch. Biochem. Biophys.* **140**, 23-28 (1970).
18. D. Kupfer, R. Partridge, and T. Munsell Jones: Alteration in the magnitude of induction of tyrosine transaminase by glucocorticoids. II. Effects of phenobarbital, *o,p'*-DDD and diethylaminoethyl diphenylpropylacetate (SKF 525A) on metabolism of triamcinolone acetate. *Arch. Biochem. Biophys.* **131**, 57-66 (1969).
19. S. Gordon and J. Morrison: The metabolic fate of triamcinolone acetate in laboratory animals. *Steroids* **32**, 25-35 (1978).
20. K. J. Kripalani, A. I. Cohen, I. Weliky, and E. C. Schreiber: Metabolism of triamcinolone acetate-21-phosphate in dogs, monkeys, and rats. *J. Pharm. Sci.* **64**, 1351-1359 (1975).
21. N. I. Chu, B. A. Amos, L. Tokés, M. L. Maddox, S. B. Matin, K. M. Hama, J. W. Patterson, P. J. Wagner, J. P. Bell, and M. D. Chaplin: Disposition of flunisolide in the rat, mouse, dog, rhesus monkey, and cynomolgus monkey. *Drug Metab. Dispos.* **7**, 81-89 (1979).
22. E. Seutter: Metabolism of systemically given corticosteroids. *Dermatologica* **151**, 129-134 (1975).
23. J. R. Florini, L. L. Smith, and D. A. Buyske: Metabolic fate of a synthetic corticosteroid (triamcinolone) in the dog. *J. Biol. Chem.* **236**, 1038-1042 (1960).
24. M. D. Chaplin, W. Rooks, E. W. Swenson, W. C. Cooper, C. Nerenberg, and N. I. Chu: Flunisolide metabolism and dynamics of a metabolite. *Clin. Pharmacol. Ther.* **27**, 402-413 (1980).
25. M. Kusama, N. Sakauchi, and S. Kumaoka: Studies of plasma levels and urinary excretion after intramuscular injection of triamcinolone acetate. *Metabolism* **20**, 590-596 (1971).

LIVER METABOLISM OF BUDESONIDE IN RAT, MOUSE AND MAN;
COMPARATIVE ASPECTS.

S. EDSBÄCKER^{O+}, P. ANDERSSON^O, C. LINDBERG^O, J. PAULSON^O,
Å. RYRFELDT^O AND A. THALÉN[∇]

^OPharmacokinetics and [∇]Organic Chemistry Laboratories, Research
and Development Department, AB Draco (Subsidiary of AB Astra),
Lund, Sweden

⁺Department of Clinical Pharmacology, University Hospital of Lund,
Lund, Sweden

ABSTRACT:

The metabolism of budesonide, (22RS)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione, was studied in 9000g supernatant fraction of livers from rat, mouse and man. The two budesonide C-22 epimers produced different metabolites. This was explained by substrate selective oxidation of the nonsymmetric 16 α ,17 α -acetal substituent. Epimer 22R gave 16 α -hydroxyprednisolone while epimer 22S produced a metabolite tentatively identified as 23-hydroxybudesonide. Otherwise budesonide followed the general metabolic pathways reported for synthetic glucocorticoids. Thus, oxidative metabolism predominated, 6 β -hydroxybudesonide and Δ^6 -budesonide being identified in all investigated species. Reductive metabolism, giving 4,5 β -dihydrobudesonide and 3,4,5 β -tetrahydrobudesonide, was most pronounced in the rat. When the rates and routes of budesonide metabolism were compared in the three species, mouse and man were most similar. This implies that the mouse is a more relevant species than the rat in studies of the pharmacology and toxicology of budesonide.

Budesonide is a potent glucocorticoid, clinically used in the local treatment of skin and respiratory diseases (1,2). In man (3-5) and laboratory animals^{1,2}(6), the drug is pharmacokinetically characterized by a large volume of distribution and a high hepatic clearance. The extensive liver biotransformation (3) contributes to the favourable ratio between local and systemic glucocorticoid effects shown for the compound (7-10). Budesonide is a 1:1 epimeric mixture, epimer 22R having 2-3 times higher topical glucocorticoid potency than epimer 22S (8). (22R)-Budesonide has approximately twice the volume of distribution, clearance and in vitro liver biotransformation rate as the 22S-epimer in man (4,11).

Several aspects of the budesonide disposition in vivo have been successfully explained by in vitro experiments performed with liver preparations³ (12,13). Two major metabolites have been identified in man: 6 β -hydroxybudesonide, formed from both epimers, and 16 α -hydroxyprednisolone, formed only from (22R)-budesonide (13).

Pharmacological and toxicological investigations performed in laboratory animals is fundamental for the clinical evaluation of new drugs. Extrapolation of animal data to man requires knowledge of species differences in pharmacokinetics. The present study was undertaken to elucidate, qualitatively and quantitatively, the budesonide metabolism. The drug epimers were separately studied, using liver 9000g supernatant fraction from rat, mouse and man.

Materials and Methods

Chemicals. Budesonide ((22RS)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione)(7), (²H₈)Budesonide ((22RS)-(22,23,23,24,24,25,25,25-²H₈)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione)(14), 6 α -Hydroxybudesonide (15), 6 β -hydroxybudesonide (15) and 1,2-dihydrobudesonide (16,17) were prepared and resolved into their 22R- and 22S-epimers as previously described.

Ethyl (22RS)-16 α ,17 α -butylidenedioxy-11 β -hydroxyandrosta-1,4-diene-3-one-17 β -carboxylate was prepared according to ref. 18.

11-Dehydrobudesonide was prepared from 250 mg of 16 α -hydroxyprednisone, 70 mg of butanal and 0.1 ml of perchloric acid (70%) in 25 ml of dioxane, in analogy with the procedure for preparation of 1,2-dihydrobudesonide (16). The crude product was purified on a Sephadex LH-20 column (85 x 2.5 cm) with heptane-chloroform-ethanol, 20:20:1, as mobile phase. The compound was precipitated from methylene chloride-petroleum ether leaving 237 mg of 11-dehydrobudesonide. M.p. 170-182°C. MS: m/z (rel. intensity) 429 (100), MH⁺; 357 (28), MH⁺-CH₃(CH₂)₂CHO; 339 (35), MH⁺-CH₃(CH₂)₂CHO-H₂O. ¹H-NMR (CDCl₃): δ 0.64 and 0.70 ppm (s, 18-H from the 22R- and 22S-epimers), 0.93 and 0.95 (t, 25-H from the 22R- and 22S-epimers), 4.0-5.4 (4 protons, 16-H, 21-H (2 protons), 22-H), 6.10 (s, 4-H), 6.21 (q, 2-H), 7.64 (d, 1-H). The 11-proton at 4.5 ppm, present in budesonide, is lost.

24-Hydroxybudesonide. To a suspension of 500 mg of 16 α -hydroxy-prednisolone in 40 ml of dioxane were added 0.2 ml of perchloric acid (70%) and a solution of 200 mg of 3-hydroxybutanal dimethyl-acetal in 10 ml of dioxane. The reaction mixture was stirred at room temperature for 5 h. Methylene chloride (300 ml) was added and the solution was washed with 10% aqueous K₂CO₃ and water, dried and evaporated. The residue was purified on a Sephadex LH-20 column (73 x 6.3 cm) using chloroform as mobile phase. The fractions 4200-4395 ml and 4770-5175 were collected, evaporated and precipitated from methylene chloride-petroleum ether yielding 25 and 64 mg of the C-22 epimers (denoted 1 and 2, respectively) of 11 β ,21-dihydroxy-16 α ,17 α -(3-hydroxybutylidene)dioxypregna-1,4-diene-3,20-dione. Epimer 1: M.p. 130-139°C. MS: m/z (rel. intensity) 447 (39), MH⁺; 429 (2), MH⁺-H₂O; 359 (14), MH⁺-CH₃CHOHCH₂CHO; 341 (100), MH⁺-CH₃CHOHCH₂CHO-H₂O. Partial ¹H-NMR spectrum is given in table 3. Epimer 2: M.p. 120-127°C. MS: m/z (rel. intensity) 447 (92), MH⁺; 429 (22), MH⁺-H₂O; 359 (37), MH⁺-CH₃CHOHCH₂CHO; 341 (100), MH⁺-CH₃CHOHCH₂CHO-H₂O.

Δ^6 -Budesonide. A solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (55 mg) in 4 ml of dry dioxane was added to 100 mg of (22RS)-21-acetoxy-11 β -hydroxy-16 α ,17 α -butylidenedioxy-4-pregnene-3,20-dione (16) in 4 ml of dry dioxane. Nitrogen (5min), dry hydrogen chloride (30 sec) and nitrogen (5 min) were passed through the solution. The mixture was stirred for 1 h and filtered. Chloroform (50 ml) was added and the solution was washed with 1 M KOH and water, dried and evaporated. The residue was purified on a Sephadex LH-20 column (82 x 2.5 cm) using chloroform as eluant, leaving 70 mg of (22RS)-21-acetoxy-11 β -hydroxy-16 α ,17 α -

butylidenedioxypregna-4,6-diene-3,20-dione. UV (ethanol): λ_{max} at 282 nm.

Selenium dioxide (150 mg) and glacial acetic acid (300 μ l) was added to a solution of 35 mg of the 4,6-diene in 35 ml of t-butanol. The reaction mixture was stirred at 75°C for 24 h under nitrogen, cooled, filtered and evaporated. The residue was dissolved in 50 ml of ethyl acetate, washed with saturated aqueous NaHCO_3 , water, cold aqueous $(\text{NH}_4)_2\text{S}$, 2 M NaOH, 2 M HCl and saturated aqueous NaHCO_3 , dried and evaporated. The crude product was purified on a Sephadex LH-20 column (87.5 x 2.5 cm) with a mixture of heptane-chloroform-ethanol, 20:20:1, as mobile phase, yielding 15 mg of (22RS)-21-acetoxy-11 β -hydroxy-16 α ,17 α ,-butylidenedioxy-pregna-1,4,6-triene-3,20-dione.

A 10% aqueous solution of K_2CO_3 (0.7 ml) was added to 15 mg of the 1,4,6-triene-21-acetate in methanol (10 ml). The reaction mixture was stirred for 10 min at 20°C under argon, diluted with water (10 ml), extracted with ethyl acetate, dried and evaporated. The residue was purified on a Sephadex LH-20 column (87 x 2.5 cm) using heptane-chloroform-ethanol, 20:20:1, as mobile phase, leaving 6 mg of (22RS)-11 β ,21-dihydroxy-16 α ,17 α -butylidenedioxy-pregna-1,4,6-triene-3,20-dione (Δ^6 -budesonide). UV (ethanol): λ_{max} at 298 nm and a shoulder at 249 nm. $^1\text{H-NMR}$ (CDCl_3): δ 0.91 ppm (t, 25-H), 0.97 (s, 18-H (22R)), 1.04 (s, 18-H (22S)), 1.43 (s, 19-H), 4.0-5.3 (5 protons, 11-H, 16-H, 21-H (2 protons), 22-H), 5.98 (s, 4-H), 6.03 (q, 2-H), 6.2-6.4 (m, 6-H and 7-H), 7.25 (d, 1-H). MS (fig 1B): m/z 429, MH^+ ; 357, $\text{MH}^+ - \text{CH}_3(\text{CH}_2)_2\text{CHO}$; 339, $\text{MH}^+ - \text{CH}_3(\text{CH}_2)_2\text{CHO} - \text{H}_2\text{O}$.

Preparative chromatography and melting point determinations of

synthesized compounds were performed as previously described (15) and ^1H -NMR spectra recorded as described below. Chemical ionization (methane) mass spectra were obtained using a direct inlet probe or a moving belt LC-MS interface (see below).

Tritiated budesonide ($[^3\text{H}]$ budesonide; (22RS)-16 α ,17 α -butylidene-dioxy-11 β ,21-dihydroxy[1,2- ^3H]pregna-1,4-diene-3,20-dione), with a specific activity of 89.4 mCi/mg, was obtained from the Radiochemical Centre, Amersham, UK. It was purified according to Andersson et al. (11), and the 22R- and 22S-epimers were separated by HPLC (see below). The radiochemical purity was estimated by HPLC at 94.5% for epimer 22R and 97.5% for epimer 22S.

16 α -Hydroxyprednisolone (11 β ,16 α ,17 α ,21-tetrahydroxypregna-1,4-diene-3,20-dione) was obtained from LARK S.p.a., Milan, Italy and 16 α -hydroxyprednisone (16 α ,17 α ,21-trihydroxypregna-1,4-diene-3,11,20-trione) from Blasinachim, Milan, Italy. Ethanol was of spectroscopic grade and water was filtered through a Millipore Super Q 4 module system. Glass distilled water was used in the LC-MS analysis. Sep-Pak C_{18} cartridges (Waters Ass. Milford, MA.) were conditioned with 5 ml of ethanol, followed by 5 ml of 0.01 M acetic acid, before use. D-Glucose-6-phosphate, NADP^+ , and glucose-6-phosphate dehydrogenase were obtained from Sigma, Sweden. ^{18}O -gas (95 atom%) was obtained from the Radiochemical Centre. A 0.1 M potassium phosphate buffer solution, pH 7.4, containing 0.2 M sucrose (buffer A) was used in all incubation experiments. A 0.01 M sodium acetate buffer solution, pH 4, (buffer B) was used in HPLC analysis. Composition of solutions are expressed in v/v terms, unless otherwise stated.

Liver Preparation. Sprague Dawley CR rats (7 of each sex) with a mean weight of 440 g for the males and 270 g for the females were obtained from Charles River Breeding Laboratories, Inc., St. Wilmington, MA. NMRI-mice (5 of each sex) with a mean weight of 21.9 g for the males and 18.0 g for the females were obtained from Anticimex, Stockholm, Sweden. Specimens of human liver were taken from one woman (age 56) and one man (age 65) shortly after circulatory arrest and stored as previously described (11).

Rats and mice were decapitated and the livers perfused in situ with ice-cold buffer A. The livers were then removed, minced, weighed and homogenized in 2.5 volumes of ice-cold buffer A with a Teflon pestle (10 strokes). The human livers were minced, weighed and then homogenized in 2.5 volumes of ice-cold buffer A with a Polytron PT 20 homogenizer for 4 x 15 s. The homogenates were centrifuged at 9000g for 20 min. Female rat liver cytosolic fraction was prepared by centrifugation of the 9000g supernatant fraction at 100,000g for 1 h. All preparations were carried out at 4°C and the homogenate fractions were then stored at -80°C. The protein concentration of the homogenates was determined according to Lowry et al. (19), using bovine serum albumine as standard.

HPLC The liquid chromatographic system consisted of an automated injector (WISP model 710 B), a model 680 gradient controller, two M 6000 A pumps, and a M 440 UV-detector ($\lambda = 254$ nm) from Waters Associates. UV-data were processed on a HP 3388 A integrator (Hewlett Packard, Avondale, PA). Analysis was performed on a 5 x 50 mm Corasil C₁₈ precolumn (Waters Associates) connected to a 5 x

200 mm analytical column, slurry packed with Nucleosil C₁₈, 5 μ m particles (Macherey-Nagel, Düren, West Germany). A gradient system was run from 32.5% to 92.5 % ethanol in buffer B in 65 min and at a flow rate of 1 ml/min. In the LC-MS analysis, a different HPLC system was used (see below).

Metabolite identification. The liver 9000g and 100,000g supernatant fractions were diluted with homogenizing buffer to a protein concentration of 3 mg/ml, and incubated with each budesonide epimer separately in the presence of MgCl₂ (10 mM), glucose-6-phosphate (40 mM), NADP⁺ (1.8 mM), and glucose 6-phosphate dehydrogenase (1.2 units/ml). Incubations for mass spectrometric analysis were started by the addition of an ethanol solution, containing a 1:1 mixture of budesonide and (²H₈)budesonide, to 20-100 ml of incubation medium, giving a final steroid concentration of 20 μ M in 1% ethanol. Incubations were continued for 1-4 h. The incubations for ¹H-NMR analysis were started by the addition of budesonide (1.5 mg, containing a trace amount of tritium labeled compound) to 350 ml of incubation medium, containing rat liver 100,000g supernatant fraction or human liver 9000g supernatant fraction. Incubations were continued for 4 h. All incubations were performed with gentle shaking at 37°C in the presence of carbogen (5/95 CO₂/O₂), ¹⁶O- or ¹⁸O-atmosphere. The technique for ¹⁸O-incubations will be reported elsewhere⁴. After incubation, proteins were precipitated by addition of ethanol and conc. hydrochloric acid. The sample, containing at most 50% ethanol and 1 M hydrochloric acid, was centrifuged at 1000g for 30 min. The supernatant fraction was diluted with water to give a final ethanol concentration of 10% and extracted on Sep-Pak C₁₈

cartridges (100 ml per cartridge). After washing of each Sep-Pak with 10 ml of 15% ethanol in 0.01 M acetic acid, the sample was eluted with 5 ml of 70% ethanol in 0.01 M acetic acid. Acetic acid (0.01 M) was added to the pooled eluates, in this way reducing the ethanol concentration to 15%. The sample was applied onto new Sep-Pak C₁₈ cartridges (50 ml per cartridge), and after washing as above, the samples were eluted with 5 ml of ethanol. After evaporation of the ethanol at 50°C under a gentle stream of nitrogen, the residue was dissolved in 25% ethanol in 0.01 M acetic acid and used for MS or NMR analysis.

Mass spectra were obtained using a Finnigan 4500 instrument (Finnigan MAT, San José, California) interfaced to an INCOS data system. A Finnigan moving belt LC-MS interface was used for the on-line LC-MS investigations. LC was performed with a Spectra Physics (San José, California) SP8700 ternary liquid chromatograph. The chromatographic column (2.1 x 100 mm), was packed with 5 μ m Nucleosil C₁₈. Chromatographic separation was achieved using a concave gradient from 28.5% to 75.0% ethanol in water during 40 min at a flow rate of 0.2 ml/min. The effluent was fed to a polyimide belt via a spray device, using nitrogen as nebulizing gas. The interface was operated at a belt speed of approximately 3.5 cm/s, a flash vaporizer temperature of 220°C and clean-up heater temperature of 270°C. The indicated ion source pressure was 0.5 torr with methane as reagent gas and an ion source temperature of 150°C. Data acquisition was started at time of injection and chemical ionization mass spectra were repetitively recorded between m/z 200 and 550. The technique will be reported in detail elsewhere⁴.

For ^1H -NMR analysis, Sep-Pak extracted samples were separated by HPLC and radioactive fractions, monitored on line with a Packard Trace 7140 detector (Packard Instruments, Downers Grove, Ill), were collected. The mobile phase was evaporated and each fraction was then dissolved in deuteriochloroform. NMR spectra were recorded on a Jeol FX 90 Q spectrometer (Jeol LTD, Tokyo, Japan) operating in the Fourier-transform mode at a frequency of 90 MHz. Chloroform (δ 7.25 ppm) was used as internal reference.

Evaluation of in vitro kinetics. Media (9000g supernatant fraction) containing 3 mg/ml of protein from male or female rat, mouse, or man, 10 mM MgCl_2 , 22.5 mM glucose-6-phosphate, 0.9 NADP+ and 1.2 units/ml glucose-6-phosphate dehydrogenase in buffer A were used. The total volume in each incubation was 15 ml. After equilibration for 10 min at 37°C, the incubations were started by the addition of 75 nmol of one of the two [^3H]budesonide C-22 epimers. Incubations were performed with gentle shaking in the presence of carbogen gas. Samples (2 ml) were removed from the incubation mixture at defined time intervals and immediately frozen on dry ice. Aliquots (2 x 50 μl) were taken at the same time intervals for recovery calculations. Immediately after thawing, 2 ml of an ethanol solution containing internal standard (10 nmol) was added to each sample. After shaking for 30 min, the samples were centrifuged for 25 min at 1000g and 3.5 ml of the supernatant fraction was evaporated at 50°C under a gentle stream of nitrogen. The residue was dissolved in 250 μl of 25% ethanol in 0.01 M acetic acid and 5 ml of 0.01 M acetic acid was added. After

storage overnight at 5°C, the sample was applied onto a Sep-Pak C₁₈ cartridge. The cartridge was washed with 5 ml of 0.01 M acetic acid and the sample eluted with 5 ml of 70% ethanol in 0.01 M acetic acid. After evaporation of the solvent at 50°C under a gentle stream of nitrogen, the sample was dissolved in 400 µl of 25% ethanol in 0.01 M acetic acid. Aliquots (200 µl) were submitted to HPLC-analysis (see above).

Aliquots were taken throughout the extraction for recovery calculations. The recovery of radioactivity after extraction and final dissolution was $91.6 \pm 4.2\%$, $82.5 \pm 3.8\%$ and $88.5 \pm 2.8\%$ (mean $\pm$ SD, n=24) for incubations with rat, mouse and human liver 9000g supernatant fraction, respectively. No decrease in extraction recovery was observed after prolonged incubation time, indicating similar recovery of parent compound and formed metabolites.

Radioactivity was measured on-line using a Packard Trace 7140 detector (Packard Instruments) or off-line by the liquid scintillation technique. The off-line measurements were performed in Optifluor using a Tricarb 460 CD (Packard Instruments) liquid scintillation counter equipped with external standard for determination of counting efficiency.

For each peak in the radio-chromatogram, the ratio of the recorded radioactivity over the UV-area of the internal standard was calculated. The relative concentration of each compound was then calculated by dividing the ratio by the ratio obtained for the parent compound at 0 min incubation time.

Results

Selected structural data and derivation of metabolites formed after budesonide incubation with liver 9000g supernatant fractions are given in table 1. HPLC retention times and MH^+ ions for some reference compounds are given in table 2. Table 3 shows partial NMR-data of some metabolites and reference compounds. Suggested metabolic pathways of budesonide, based on the data in tables 1-3, are shown in fig. 1.

Reductive pathways of metabolism. The UV-spectrum of budesonide is characterized by strong absorption at 254 nm ($\epsilon_{254 \text{ nm}} = 1.2 \times 10^4 \text{ cm}^{-1}M^{-1}$, $\lambda_{\text{max}} = 242 \text{ nm}$ (20)). This is due to the presence of the conjugated α, β -unsaturated ketone in the A-ring (21). A-ring reduction decreases UV-absorptivity at 254 nm and increases the molecular weight by at least 2 u. Metabolites I-III showed these characteristics (table 1).

Selected NMR signals of metabolites I and II are given in table 3. The NMR spectrum of metabolite I showed: 1/ no conjugated 3-keto- $\Delta^{1,4}$ structure; 2/ the appearance of a proton at δ 4.1-4.6 ppm (interpreted as a proton signal of the reduced 3-keto group); and 3/ the appearance of two protons at δ 5.5-5.9 ppm (interpreted as the protons at the 1,2-double bonded carbons). Taken together, NMR- (table 3), UV- and MS-data (table 1) of metabolite I strongly indicate hydrogenation of the 3,4 and 5 positions of budesonide. The NMR spectrum of metabolite II showed: 1/ two doublets at δ 5.94 and 7.02 ppm ($J=10 \text{ Hz}$); 2/ no signal at δ 6.03 ppm (C-4 proton in budesonide); and 3/ loss of the meta coupling of the

C-2 proton. This strongly indicates an intact 1,2 double bond and a reduction at the 4,5-position. The shifts of the C-1 and C-2 protons of metabolite II were almost identical to those reported for (5 β ,11 β ,16 β)-11,21-dihydroxy-2'-methyl-5 β -pregn-1-ene[17,16-d]oxazole-3-20-dione, a 5 β -reduced metabolite of deflazacort (22) (partial NMR spectrum is given in table 3). Thus, NMR- (table 3), UV- and MS-data (table 1) strongly indicate that metabolite II is 4,5 β -dihydrobudesonide (fig. 1). The observation that metabolite II could be generated from both budesonide and the 3,4,5-hydrogenated metabolite I (see below) suggests that metabolite I is 3,4,5 β -tetrahydrobudesonide (fig. 1).

Metabolite III could not be sufficiently purified to allow final identification. UV- and MS-data (table 1) showed reduction of at least one of the A-ring double bonds. The MH^+ ion was found at m/z 449 and 451 after incubation in ^{16}O - and ^{18}O -atmosphere, respectively. This indicates monooxygenation of the steroid nucleus. Mass spectral data (not shown) indicated intact 17 β and 16 α ,17 α -acetal side chains.

Tritium labeled budesonide C-22 epimers were incubated with rat liver 100,000g supernatant fraction (cytosolic fraction) in the presence of NADPH. 3,4,5 β -Tetrahydrobudesonide and 4,5 β -dihydrobudesonide were formed from both epimers. The compounds were isolated and separately incubated with rat liver 9000g supernatant fraction. 4,5 β -Dihydrobudesonide as well as several more polar metabolites were formed from 3,4,5 β -tetrahydrobudesonide. The polar compounds could be tentatively identified by mass spectrometry and UV spectroscopy as A-ring reduced plus hydroxylated,

or acetal group cleaved, budesonide derivatives (see below). Incubation of 4,5 β -dihydrobudesonide gave the same polar metabolites, but no 3,4,5 β -tetrahydroxybudesonide could be detected.

Oxidative pathways of metabolism. The MS-data of metabolite IV (table 1) indicated dehydrogenation of budesonide. Comparison with authentic Δ^6 -budesonide showed a similar mass spectrum (fig. 2) and similar HPLC retention time (table 2). The identity of metabolite IV was thus confirmed as Δ^6 -budesonide (fig. 1).

Metabolites V and VI (table 1) have previously been identified (13) as 6 β -hydroxybudesonide and 16 α -hydroxyprednisolone, respectively (fig. 1). 16 α -Hydroxyprednisolone is formed by 16 α ,17 α -acetal group cleavage of (22R)-budesonide. The reaction requires oxidative and hydrolytic enzyme activity⁵.

The mass spectrum of metabolite VII showed an MH⁺ ion at m/z 447 and loss of one of the deuterium atoms (table 1). When the metabolite was formed under ¹⁸O-atmosphere, the MH⁺ ion increased by 2 u (data not shown). These results indicate hydroxylation of the 16 α ,17 α -acetal group. HPLC analysis showed that metabolite VII did not have the same retention time as authentic 24-hydroxybudesonide (table 2). NMR spectrum of metabolite VII (table 3) revealed a downfield shift by 0.32 ppm of the C-25 protons and the absence of a signal at δ 3.8-4.1 ppm (C-24 protons) when compared with the spectrum of 24-hydroxybudesonide (table 3). Instead a signal appeared at δ 5.1-5.4 ppm. The introduction of a hydroxyl substituent in the 25-position should result in loss of the 25-CH₃ proton signals at δ 0.9 ppm which was not the case. NMR data of

the acetal hydroxylated metabolite thus excludes hydroxylation in the 24 and 25 positions. Hydroxylation in the 22-position results in the formation of a hemiorthoester. This is a chemically labile entity and will rearrange to 16 α - or 17 α -butyryloxyprednisolone⁵. The mass spectrum of metabolite VII did not conform with such an ester (data not shown). Consequently, hydroxylation in the 22, 24, or 25 positions can be excluded and we therefore suggest that metabolite VII is 23-hydroxybudesonide (fig. 1).

Species and sex differences in budesonide metabolism. Figs. 3A and 3B show the biotransformation rates of (22R)- and (22S)-budesonide. The rates of formation of some major metabolites are illustrated in figs. 3C and 3D. 16 α -Hydroxyprednisolone was not formed from (22S)-budesonide. Relative concentrations of (22R)- and (22S)-budesonide and main metabolites, after 40 and 120 min incubation time, are given in tables 4 and 5. Biotransformation half-lives of the parent compounds are also given in tables 4 and 5.

A sex difference in the metabolism of budesonide was seen in the rat. The formation rate of major metabolites also differed. 6 β -Hydroxybudesonide was the quantitatively most important metabolite from both epimers in male rat liver. In the female rat, A-ring reduction was most important for (22R)-budesonide and acetal side chain hydroxylation for (22S)-budesonide. Marginal sex difference in budesonide metabolism was found in the mouse and human livers. In both species, 16 α -hydroxyprednisolone was the quantitatively most important metabolite from (22R)-budesonide and 6 β -hydroxybudesonide from the 22S-epimer.

Discussion

Most of the budesonide metabolites identified in the present study represent metabolic pathways common to other glucocorticoids.

A-ring reduction, the major metabolic event of cortisol (23), has been demonstrated for several 3-keto- $\Delta^{1,4}$ -diene glucocorticoids (22,24,25). NADPH-dependent Δ^4 -5 β -reductase and 3 α - and 3 β -hydroxysteroid dehydrogenases are present in rat liver microsomal and cytosolic fractions (23,26,27). While the Δ^4 -reduction is irreversible (23), the equilibrium of the reversible dehydrogenases are governed, inter alia, by cofactor concentration (26,28). 3,4,5 β -Tetrahydrobudesonide and 4,5 β -dihydrobudesonide (I and II in fig. 1), and unidentified oxidized metabolites generated from these A-ring reduced compounds, were abundant in the rat.

Metabolite III, which is A-ring reduced plus oxygenated, was formed primarily in liver 9000g supernatant fraction from man, but could also be detected in the mouse. A similar biotransformation has been shown for deflazacort (22) in man.

Metabolic oxidation is quantitatively important for most synthetic glucocorticoids, and 6 β -hydroxylation is generally a major metabolic pathway³ (22,29-33). Dehydrogenation of the B-ring to the corresponding Δ^6 or Δ^7 derivative has been demonstrated for flunisolide (34), triamcinolone acetonide (35), and deflazacort (22). Δ^6 -Budesonide and 6 β -hydroxybudesonide (metabolites IV and V in fig. 1) were formed from both budesonide epimers in all investigated liver preparations.

The biotransformation of the nonsymmetric 16 α ,17 α -acetal substituent of budesonide was extensive. This is in contrast to the symmetric 16 α ,17 α -acetonides, which are considered to have a metabolically stable 16 α ,17 α -acetal group (13,31,33). The acetal cleavage of (22R)-budesonide, giving 16 α -hydroxyprednisolone (metabolite VI in fig. 1), was prominent in the mouse (more than 70% conversion for (22R)-budesonide), and in man (50% conversion), but not in the rat (5-10% and 15-20 % conversion in female and male rat, respectively)(fig. 3C). 16 α -Hydroxyprednisolone has been shown to be the major budesonide metabolite in man after i.v. administration³. (22S)-Budesonide was not biotransformed by acetal cleavage. Instead 23-hydroxylation of (22S)-budesonide (metabolite VII in fig. 1) occurred, especially in rat liver.

Substrate selective oxidation of the 16 α ,17 α -acetal group is probably the major reason for the difference in biotransformation rate between the two budesonide epimers (tables 4-5). (22R)-budesonide was biotransformed five times as fast as the 22S-epimer in the mouse, about two times faster in man and at about equal rate in the rat.

Species differences were observed in the liver metabolism of budesonide (fig. 3). In the rat, there was a marked sex difference in budesonide biotransformation rate, previously demonstrated by Andersson et al. (12). The greater 6 β -hydroxylase activity of the male rat liver seems to be the major reason to the observed differences. In the metabolism of endogenous steroids, male rats have reduced activity towards A-ring reduction together with an

increased hydroxylase activity (27). This difference in metabolism between sexes is possibly unique for the rat (36). Accordingly, marginal sex difference in budesonide metabolism was seen in the mouse and human livers. The metabolism in mouse and man was quantitatively and qualitatively similar, being extensive and predominantly oxidative.

The glucocorticoid receptor binding affinity (RBA) may be used to express the intrinsic glucocorticoid activity (37). The RBA values and biological activities of 6 β -hydroxybudesonide and 16 α -hydroxy-prednisolone are less than 1% of that of budesonide (37). The RBA value of budesonide hydroxylated in the acetal side chain (24-hydroxybudesonide) is less than 3% of that of budesonide⁶. An intact Δ^4 -3-one group and a saturated B-ring of glucocorticoids are essential for retaining a high glucocorticoid activity (38). Taken together, the in vitro biotransformation of budesonide leads to the formation of metabolites with strongly reduced glucocorticoid potency.

It may be concluded that budesonide is rapidly biotransformed in liver homogenates from man, mouse and male rat. Apart from the metabolic pathways involving the nonsymmetric 16 α ,17 α -acetal moiety, which are unique for budesonide, the metabolism is similar to that of other synthetic glucocorticoids. Of the two animal species studied, the biotransformation of budesonide in the mouse is most similar to that of man. Thus, from a metabolic point of view, the mouse seems to be a more relevant species than the rat when extrapolating pharmacological and/or toxicological data to man.

Acknowledgements

We are indebted to Mr. B. Nylander and Mr. L.-I. Wickström for their skillful assistance with the experimental synthetic work. We also wish to thank Miss G. Schwabe for recording ^1H -NMR-spectra of the isolated metabolites, and assisting in the interpretation of these spectra.

Footnotes:

Reprint requests to: Staffan Edsbäcker, M.S., Pharmacokinetic laboratory, AB Draco, Box 34, S-22101 Lund, Sweden.

- 1 P. Andersson, and Å. Ryrfeldt, pharmacokinetics of [³H]budesonide in the mouse, manuscript in preparation.
- 2 P. Andersson, S. Edsbäcker, E. Nilsson, and Å. Ryrfeldt, pharmacokinetics of [³H]budesonide in the rat, preliminary data.
- 3 S. Edsbäcker, and Å. Ryrfeldt, Systemic elimination of the topical glucocorticoid budesonide in man; metabolic aspects, manuscript in preparation.
- 4 C. Lindberg, S. Edsbäcker, and J. Paulson, manuscript in preparation.
- 5 S. Edsbäcker, P. Andersson, C. Lindberg, and Å. Ryrfeldt, submitted.
- 6 B. Axelsson, unpublished data.

References

1. S.P.Clissold and R.C.Heel: Budesonide - A preliminary review of its pharmacodynamic properties and therapeutic efficacy in asthma and rhinitis. *Drugs* 28, 485-518 (1984).
2. A.Heijer, G.Hesser, P.Holm and L.Salde: Comparison between two non-halogenated glucocorticoid ointments in psoriasis. *J.Int.Med.Res.* 9, 239-247 (1981).
3. Å.Ryrfeldt, P.Andersson, S.Edsbäcker, M.Tönnesson, D.Davies and R.Pauwels: Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur.J.Respir.Dis.* 63, Suppl. 122, 86-95 (1982).
4. Å.Ryrfeldt, S.Edsbäcker and R.Pauwels: Kinetics of the epimeric glucocorticoid budesonide. *Clin.Pharmacol.Ther.* 35, 525-530 (1984).
5. S.Edsbäcker, K.E.Andersson and Å.Ryrfeldt: Nasal bioavailability and systemic effects of the glucocorticoid budesonide in man. *Eur.J.Clin.Pharmacol.* 29, 477-481 (1985).
6. Å.Ryrfeldt, M.Tönnesson, E.Nilsson and A.Wikby: Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by HPLC. *J.Steroid Biochem.* 10, 317-324 (1979).

7. A.Thalén and R.Brattsand: Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with high local activity. *Arzneim.Forsch.Drug Res.* 29, 1687-1690 (1979).
8. R.Brattsand, A.Thalén, K.Roempke, L.Källström and E.Gruvstad: Influence of 16 α ,17 α -acetal substitution and steroid nucleus fluorination on the topical to systemic activity of glucocorticoids. *J.Steroid Biochem.* 16, 779-786 (1982).
9. R.Brattsand, A.Thalén, K.Roempke, L.Källström and E.Gruvstad: Development of new glucocorticoids with a very high ratio between topical and systemic activities. *Eur.J.Respir.Dis.* 63, Suppl. 122, 62-73 (1982).
10. S.Å.Johansson, K.E.Andersson, R.Brattsand, E.Gruvstad and P.Hedner: Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. *Eur.J.Clin.Pharmacol.* 22, 523-529 (1982).
11. P.Andersson, S.Edsbäcker, Å.Ryrfeldt and C.von Bahr: In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J.Steroid Biochem.* 16, 787-795 (1982).

12. P.Andersson, R.Brattsand, S.Edsbäcker, L.Källström and Å.Ryrfeldt: Biotransformation rate (in vitro) and systemic potency (in vivo) of the topical glucocorticoid budesonide in male and female rats. J.Steroid Biochem. 17, 703-706 (1982).
13. S.Edsbäcker, S.Jönsson, C.Lindberg, Å.Ryrfeldt and A.Thalén: Metabolic pathways of the topical glucocorticoid budesonide in man. Drug Metab.Disp. 11, 590-596 (1983).
14. A.Thalén: Epimers of budesonide and related corticosteroids. II. Structure elucidation by mass spectrometry. Acta Pharm.Suec. 19, 327-354 (1982).
15. A.Thalén and L.-I.Wickström: Synthesis and structure elucidation of 6 α - and 6 β -hydroxylated budesonide and related corticosteroids. Acta Pharm.Suec. 21, 145-162 (1984).
16. A.Thalén, R.Brattsand, and E.Gruvstad: The synthesis and pharmacological properties of some 16 α ,17 α -acetals of 16 α -hydroxycortisone, 16 α -hydroxyprednisolone and fluorinated 16 α -hydroxyprednisolones. Acta Pharm.Suec. 21, 109-124 (1984).
17. US patent 4,404,200.
18. European patent specification 143 764.

19. O.H.Lowry, N.J.Rosebrough, A.H.Farr and R.J.Randall: Protein measurement with the folin phenol reagent. *J.Biol.Chem.* 193, 265-275 (1951).
20. A.Wikby, L.Nilsson and G.Hällsås: Separation of epimers of budesonide and related corticosteroids by reversed bonded-phase liquid chromatography. *J.Chromatogr.* 157, 51-64, (1978).
21. R.M.Silverstein, G.C.Bassler and T.C.Morrill: Spectrometric identification of organic compounds, 3d edition, p. 243. John Wiley and son Inc, 1974.
22. E.Martinelli, P.Ferrari, A.Ripamonti, G.Tuan, A.Perazzi and A.Assandri: Metabolism of deflazacort in rat, dog and man. *Drug Metab.Disp.* 7, 335-339 (1979).
23. A.F.Clark: Steroid Δ^4 -reductases; their physiological role and significance. In "Steroid Biochemistry" (R.Hobkirk, ed.), p.5. CRC Press, Boca Raton, 1979.
24. R.Lambe, S.Hudson, D.O'Kelly and A.Darragh: The metabolism of fluocortolone in the ram. *Ir.J.Med.Sci.* 147, 393-403 (1979).
25. A.Vermeulen: The metabolism of 4-¹⁴C prednisolone. *J.Endocrin.* 18, 278-291 (1959).

26. D.B.Gower: Catabolism and excretion of steroids. In "Biochemistry of steroid hormones" (H.L.J. Makin, ed.), pp 149-184, Blackwell Scientific Publications, London, 1975.
27. H.Schrievers: Factors regulating the metabolism of steroids. Vitamins Horm. 25, 271-314 (1967).
28. P.Jouan and S.Samperez: Metabolism of steroid hormones in the brain. In "The endocrine function of the brain" (Motta, ed.), p.103. Raven Press, New York, 1980.
29. A.Assandri, P.Ferrari, A.Perazzi, A.Ripamonti, G.Tuan and L.Zerilli: Disposition and metabolism of a new steroidal anti-inflammatory agent deflazacort in cynomolgus monkeys. Xenobiotika 13, 185-196 (1983).
30. I.Hornke, F.Cavagna, O.Christ, H.W.Fehlaber, H.M.Kellner and J.Sandow: Pharmakokinetik und Metabolismus von Desoximetason. Arzneim.Forsch.Drug Res. 30, 47-54 (1980).
31. K.J.Kripalani, A.I.Cohen, I.Weliky and E.C.Schreiber: Metabolism of triamcinolone acetone-21-phosphate in dogs, monkeys and rats. J.Pharm.Sci. 64, 1351-1359 (1975).
32. D.S.Skrabalak and G.A.Maylin: Dexamethasone metabolism in the horse. Steroids 39, 233-244 (1982).

33. N.I.Chu, B.A.Amos, L.Tökés, M.L.Maddox, S.B.Martin, K.M.Hama, J.W.Patterson, P.J.Wagner, J.P.Bell and M.D.Chaplin: Disposition of flunisolide in the rat, mouse, dog, rhesus monkey, and cynomolgus monkey. *Drug Metab.Disp.* 7, 81-89 (1979).
34. P.J.Teitelbaum, N.I.Chu, D.Cho, L.Tökés, J.W.Patterson, P.J.Wagner and M.D.Chaplin: Mechanism for the oxidative defluorination of flunisolide. *J.Pharmacol.Exp.Ther.* 218, 16-22 (1981).
35. S.Gordon and J.Morrison: The metabolic fate of triamcinolone acetonide in laboratory animals. *Steroids* 32, 25-35 (1978).
36. R.Kato: Sex-related differences in drug metabolism. *Drug Metab.Rev.* 3, 1-32 (1974).
37. E.Dahlberg, A.Thalén, R.Brattsand, J.Å.Gustafsson, U.Johansson, K.Roempke and T.Saartok: Correlation between chemical structure, receptor binding and biological activity of some novel, highly active 16 α ,17 α -acetal substituted glucocorticoids. *Mol.Pharm.* 25, 70-78 (1984).
38. I.E.Bush: Metabolic factors affecting the biological activities of steroid hormones. *Pharm.Rev.* 14, 361-401 (1962).

TABLE 1.

Selected structural data and derivation of budesonide metabolites.

Incubation was performed in liver 9000g supernatant fraction from rat, mouse and man. The retention times of the metabolites are given relative to (22S)-budesonide.

Metabolite	HPLC relative retention		Deuterium labelling	UV-response ^b	MH ⁺ ion (m/z)	Derivation
	22R	22S				
I	1.07	1.15	intact ^a	-	435	rat
II	1.13-1.15	1.19	"	(+)	433	rat, man
III	0.80-0.81	0.83-0.88	"	-	449	mouse, man
IV	0.97-0.98	1.02-1.03	"	+	429	rat, mouse, man
V	0.58-0.62	0.63-0.65	"	+	447	rat, mouse, man
VI	0.21-0.24	-	lost	+	377	rat, mouse, man
VII	-	0.45-0.48	7 u apart	+	447	rat, mouse, man

^aIntact deuterium labelling implies isotope peaks 8 u apart.

^bUV-absorption was measured at 254 nm. + = UV-response comparable to that of budesonide,

(+) = UV-response reduced as compared to that of budesonide, and - = no detectable UV-response.

TABLE 2.

HPLC retention times relative to that of (22S)-budesonide, and
m/z values of the MH⁺ ions of some authentic reference
compounds.

Compound		HPLC relative retention	MH ⁺ ion (m/z)
16 α -Hydroxyprednisolone		0.23	377
6 α -Hydroxybudesonide	epimer 22R	0.40	447
	22S	0.42	"
24-Hydroxybudesonide	epimer 1	0.48	447
	2	0.58	"
6 β -Hydroxybudesonide	epimer 22R	0.63	447
	22S	0.67	"
11-Dehydrobudesonide	epimer 1	0.81	429
	2	0.83	"
Budesonide	epimer 22R	0.95	431
	22S	1.00	"
Δ^6 -Budesonide	epimer 1	0.96	429
	2	1.01	"
1,2-Dihydrobudesonide	epimer 22R	1.00	433
	22S	1.06	"

TABLE 3.

Partial $^1\text{H-NMR}$ spectra of (22R)-budesonide, 4,5 β -dihydrodeflazacort, 24-hydroxybudesonide (epimer 1) and budesonide metabolites I, II and VII.

All compounds were dissolved in deuteriochloroform. Chloroform (δ 7.25) was used as internal reference.

Compound	Chemical shifts (ppm) for protons at position														
	1	2	4	11	16	18	19	21	22	23	24	25			
(22R)-Budesonide	7.21	6.27	6.03	4.1-4.6	4.89	0.92	1.46	4.1-4.6	4.54	n.d.	n.d.	0.92			
4,5 β -Dihydrodeflazacort ^a	7.01	5.94		4.2-4.6		0.93	1.46	4.2-4.6							
24-Hydroxybudesonide (epimer 1)	7.2	6.27	6.02	4.4-4.6	5.2-5.4	0.99	1.44	4.1-4.7	5.3	n.d.	3.8-4.1	1.19			
Metabolite I	5.5-5.9	5.5-5.9	n.d.	4.1-4.6	4.88	0.84	1.28	4.1-4.6	4.54	n.d.	n.d.	0.96			
Metabolite II	7.02	5.94	n.d.	4.1-4.6	4.88	n.d.	n.d.	4.1-4.6	4.54	n.d.	n.d.	n.d.			
Metabolite VII	7.20	6.27	6.02	4.1-4.7	5.1-5.4	0.99	n.d.	4.1-4.7	5.1-5.4	5.1-5.4	n.d.	0.87			

^a data from reference 22.

n.d. = not determined.

TABLE 4.

Relative concentrations of budesonide and main metabolites formed after 40 and 120 min incubation of (22R)-budesonide with liver 9000g supernatant fraction from male and female rat, mouse and man.
 Relative concentration of parent compound at 0 min incubation time was set to 1.00. The estimated $t_{1/2}$ of the parent compound is given for 40 min incubation time unless otherwise stated.

Gender	male			female		
	rat	mouse	man	rat	mouse	man
Species						
Incubation time (min)	40 120	40 120	40 120	40 120	40 120	40 120
A-ring reduced metabolites						
Metabolite III	BLQ BLQ	0.07 0.06	0.07 0.11	BLQ BLQ	0.06 0.04	0.10 0.05
Other A-ring reduced metabolites	0.27 0.30	BLQ BLQ	BLQ BLQ	0.10 0.25	BLQ BLQ	BLQ BLQ
Oxidized metabolites						
δ^6 -Budesonide	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ
6 β -Hydroxybudesonide	0.39 0.37	0.17 0.11	0.11 0.08	0.02 0.07	0.14 0.05	0.09 BLQ
16 α -Hydroxyprednisolone	0.15 0.20	0.72 0.72	0.43 0.52	0.05 0.10	0.78 0.73	0.53 0.49
23-Hydroxybudesonide	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ
Budesonide	0.21 0.04	BLQ BLQ	0.22 BLQ	0.82 0.58	BLQ BLQ	BLQ BLQ
Budesonide $t_{1/2}$ (min)	26	6 ^a	18	206	4 ^a	8 ^a

BLQ= below limit of quantitation.

^aCalculated after 20 min incubation.

TABLE 5.

Relative concentrations of budesonide and main metabolites formed after 40 and 120 min incubation of (22S)-budesonide with liver 9000g supernatant fraction from male and female rat, mouse and man.

Relative concentration of parent compound at 0 min incubation time was set to 1.00. The estimated $t_{1/2}$ of the parent compound is given for 40 min incubation time unless otherwise stated.

Gender	male			female		
	rat	mouse	man	rat	mouse	man
Species						
Incubation time (min)	40 120	40 120	40 120	40 120	40 120	40 120
A-ring reduced metabolites						
Metabolite III	BLQ BLQ	0.03 0.03	0.12 0.25	BLQ BLQ	0.03 0.03	0.21 0.18
Other A-ring reduced metabolites	0.10 0.12	BLQ BLQ	BLQ BLQ	0.04 0.06	BLQ BLQ	BLQ BLQ
Oxidized metabolites						
Δ^6 -Budesonide	BLQ BLQ	0.12 0.11	BLQ BLQ	BLQ BLQ	0.09 0.04	BLQ BLQ
6 β -Hydroxybudesonide	0.39 0.47	0.22 0.34	0.20 0.29	0.02 0.11	0.29 0.39	0.30 0.27
16 α -Hydroxyprednisolone	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ
23-Hydroxybudesonide	0.22 0.20	0.14 0.25	0.07 0.09	0.19 0.36	0.19 0.26	0.12 0.12
Budesonide	0.37 BLQ	0.38 0.12	0.46 0.08	0.83 0.49	0.22 0.04	0.14 BLQ
Budesonide $t_{1/2}$ (min)	29	29	36	169	18	14

BLQ= below limit of quantitation.

^aCalculated after 20 min incubation.

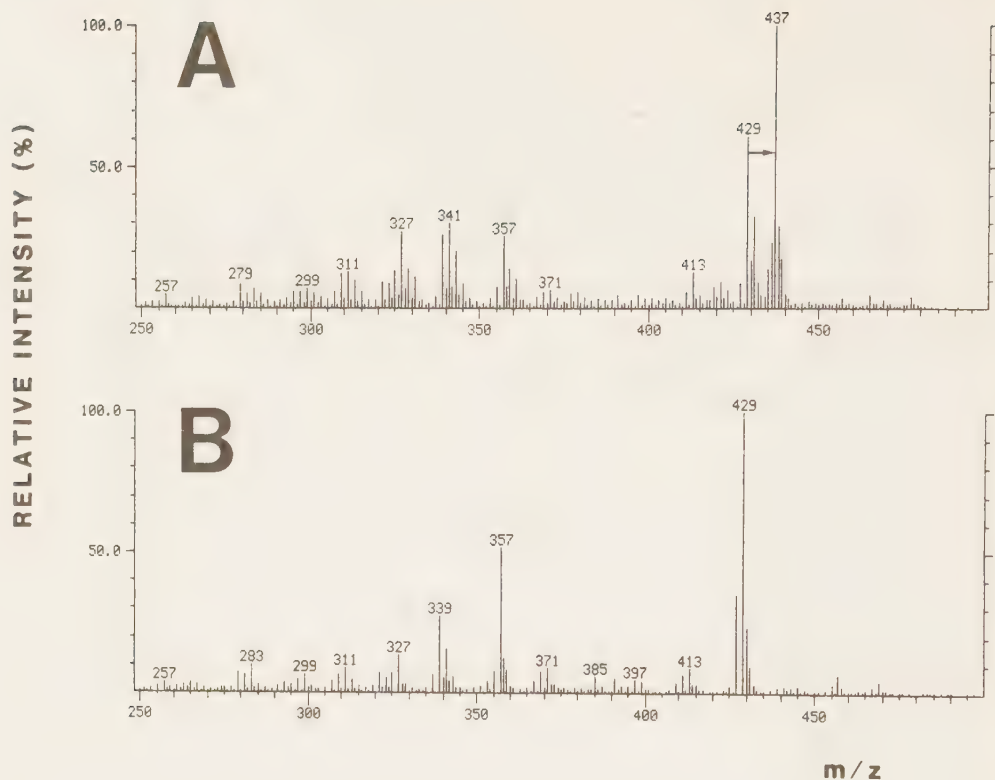


FIG. 1. Chemical ionization (methane) mass spectra of metabolite IV (A) and authentic Δ^6 -budesonide (B).

Metabolite IV was obtained by incubation of epimer (22R) of budesonide and ($^2\text{H}_8$)budesonide with mouse liver 9000g supernatant fraction. Arrow indicate deuterium containing ion.

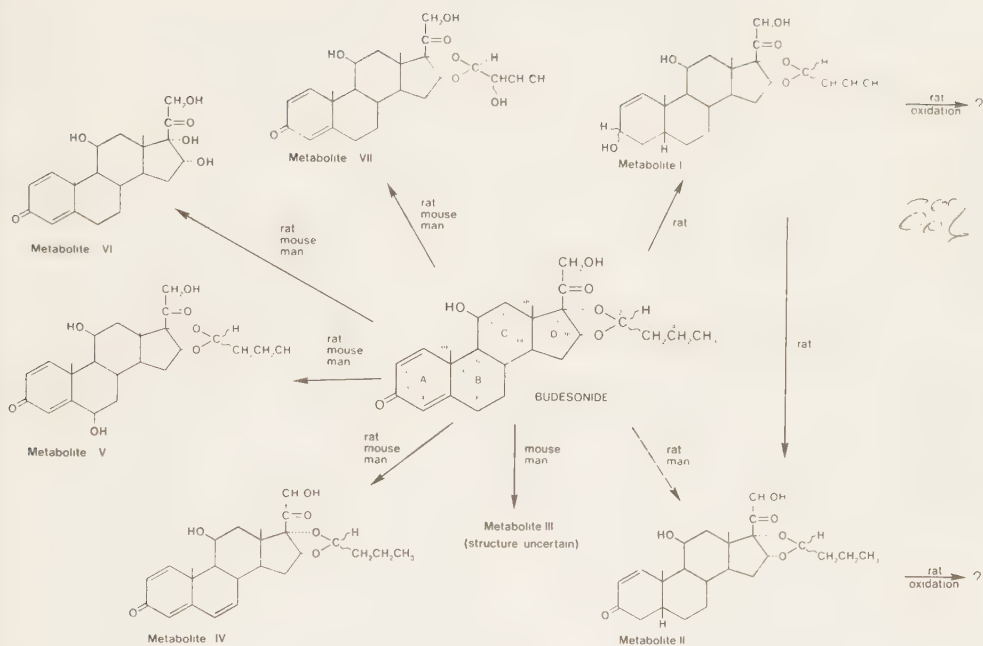


FIG. 2. Suggested metabolic pathways for budesonide in liver 9000g supernatant fraction from rat, mouse and man.

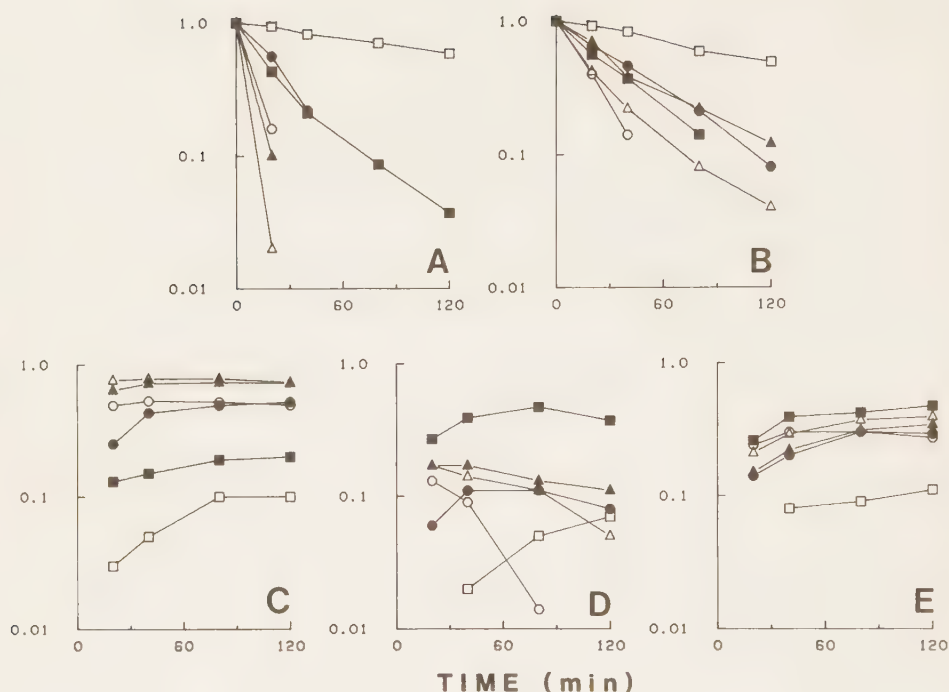


FIG. 3. Relative concentration of budesonide epimers (22R) (A) and (22S) (B), 16 α -hydroxyprednisolone (C) and 6 β -hydroxybudesonide epimers (22R) (D) and (22S) (E) after incubation of [3 H]budesonide with liver 9000g supernatant fraction from rat (■□), mouse (▲△), and man (●○).

Filled symbols indicate male and open symbols indicate female livers. 16 α -Hydroxyprednisolone and (22R)-6 β -hydroxybudesonide were formed from (22R)-budesonide. (22S)-6 β -hydroxybudesonide but no 16 α -hydroxyprednisolone was formed from (22S)-budesonide.



METABOLIC ACETAL SPLITTING OF BUDESONIDE - A NOVEL INACTIVATION
PATHWAY FOR TOPICAL GLUCOCORTICIDS.

S. EDSBÄCKER^{o+}, P. ANDERSSON^o, C. LINDBERG^o, Å. RYRFELDT^o
AND A. THALÉN^v

^oPharmacokinetics and ^vOrganic Chemistry Laboratories, Research
and Development Department, AB Draco (Subsidiary of AB Astra),
Lund, Sweden

⁺Department of Clinical Pharmacology, University Hospital of
Lund, Lund, Sweden

ABSTRACT:

Topical glucocorticoids usually have a high intrinsic glucocorticoid potency and may after systemic uptake induce side effects. The systemic inactivation of budesonide is rapid due to extensive liver biotransformation. The major metabolic pathway, 16 α ,17 α -acetal splitting, is unique for budesonide within this group of compounds. This biotransformation is catalyzed by microsomal monooxygenases and proceeds via hydroxylation and subsequent rearrangement to an intermediary ester. The ester is cleaved by hydrolysis to 16 α -hydroxyprednisolone and butyric acid. The hydrolysis product 16 α -hydroxyprednisolone has strongly reduced glucocorticoid activity.

Budesonide (fig. 1) is a potent glucocorticoid, used clinically in the local treatment of skin (1) and respiratory diseases of an inflammatory and/or allergic ethiology (2). The drug deviates structurally from the common topical $16\alpha,17\alpha$ -acetonide glucocorticoids by a new type of nonsymmetric $16\alpha,17\alpha$ -acetal substituent with a 1:1 ratio between epimers 22R and 22S (3). This chemical modification not only improves the topical anti-inflammatory potency at least five-fold as compared with the symmetric $16\alpha,17\alpha$ -acetonide analogue (4), but also provides additional sites for metabolic inactivation in the liver (5,6). The major budesonide metabolite, both in vitro in human liver preparations¹ (5,6) and in plasma after i.v. administration to healthy subjects¹, is 16α -hydroxyprednisolone (fig. 1). The glucocorticoid potency of this metabolite is less than 1% of that of the parent compound (7). In the pharmacological and toxicological evaluation of new chemical entities, the metabolism is always of importance. Experiments were therefore undertaken to in more detail elucidate the mechanism of the metabolic $16\alpha,17\alpha$ -acetal splitting, which involves only the 22R-epimer of budesonide (5,6). This unique and highly efficient inactivation pathway contributes in a fundamental way to the favourable ratio between local therapeutic effect and systemic side effects noted for the drug (8,9).

Material and methods

Chemicals. Budesonide ((22RS)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione) and (²H₈)budesonide ((22RS)-(22,23,23,24,24,25,25,25-²H₈)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione, were prepared and resolved into their 22R- and 22S-epimers as previously described (3,10).

16 α -Butyryloxy prednisolone. To a suspension of 0.75 g of 16 α -hydroxyprednisolone in dry dioxane (7.5 ml) was added trimethylorthobutyrate (0.75 ml), anhydrous methanol (6 drops) and 70% of perchloric acid (2 drops). After 5 min the reaction mixture was essentially clear and filtered into a flask containing 0.75 ml of pyridine. Water (25 ml) was added followed by solid NaCl to saturation. The upper phase was separated, dissolved in methylene chloride and dried over Na₂SO₄. The solvents were evaporated and the residue precipitated from methylene chloride-petroleum ether leaving 0.80 g of 16 α ,17 α -(1-methoxy)butylidenedioxy-11 β ,21-dihydroxypregna-1,4-diene-3,20-dione.

To a solution of this compound in 40 ml of methanol was added 6.5 ml of 2M HCl. After 5 min at room temperature, the reaction mixture was neutralized with NaHCO₃ and evaporated. Water (50 ml) was added and the solution was extracted with ethyl acetate, dried and evaporated. The residue was purified by chromatography on a Sephadex LH-20 column, using chloroform as

mobile phase, yielding 0.41 g of a solid product after precipitation from methylene chloride-petroleum ether. The purity as estimated by HPLC (μ Bondapak C₁₈; 45% ethanol (v/v) in water) was 97.7%. MS (fig. 3C): m/z 447, MH^+ ; 429, MH^+-H_2O ; 387, $MH^+-HCOCH_2OH$; 369, $MH^+-HCOCH_2OH-H_2O$; 359, $MH^+-CH_3(CH_2)_2COOH$; 341, $MH^+-CH_3(CH_2)_2COOH-H_2O$; 329, $MH^+-CH_3(CH_2)_2COOH-HCHO$. ^{13}C -NMR (DMSO- d_6): δ (ppm) 65.9 (C-21), 74.7 (C-16) and 87.7 (C-17). A comparison with the corresponding carbon resonances of 16 α -hydroxyprednisolone, 66.4, 71.2 and 87.5 ppm, respectively, shows a deshielding of only the C-16 atom in the product of a magnitude which is in agreement with acylation of the 16 α -hydroxy group (11).

The downfield shift in 1H -NMR (C^2HCl_3) of the C-16 proton, from δ 4.90 in 16 α -hydroxyprednisolone to δ 5.71 ppm in the product and to 5.72 in 16 α ,21-diacetoxy-11 β ,17 α -dihydroxypregna-1,4-diene-3,20-dione, further confirms the structure of the product as 16 α -butyryloxyprednisolone.

NMR spectra were recorded on a Jeol FX 90Q spectrometer (Jeol LTD, Tokyo, Japan) operating in the Fourier-transform mode at a frequency of 89.6 MHz (1H -NMR) and 22.5 MHz (^{13}C -NMR). Tetramethylsilane and chloroform (δ 77.1 ppm), respectively, were used as internal references.

[1,2- 3H]Budesonide (specific activity 43 Ci/mmol, [22,23,24,25- 3H]budesonide (68 Ci/mmol), [1,2,3,4- 3H]butyraldehyde (50 Ci/mmol), [1- ^{14}C]butyric acid (55 mCi/mmol) and ^{18}O -gas (95

atom %) were obtained from The Radiochemical Centre, Amersham, England. The tritium labeled epimers of budesonide were separated and purified (6). The radiochemical purity, determined by HPLC, was >93% in both cases.

A solution of 2,4-dinitrophenylhydrazine (2,4-DNPH, Merck, Darmstadt, Germany) in 2 M HCl was prepared and purified according to Fung et al. (10). Phenylmethylsulfonylfluoride (PMSF), bis(p-nitrophenyl)phosphate (BPNPP), D-glucose-6-phosphate, NADP^+ , glucose-6-phosphate dehydrogenase and carboxylic ester hydrolase from porcine liver were obtained from Sigma, Sweden. Trimethylsilylimidazole (TMSI) was obtained from Merck. Diethyl ether, ethanol and methanol were of analytical grade. Butyraldehyde was freshly distilled before use. Water was filtrated through a Millipore Super Q 4 module system. All HPLC solvents were helium-degassed prior to use. A 0.1 M potassium phosphate buffer solution, pH 7.4, containing 0.2 M sucrose was used in all incubation experiments.

Incubation experiments. Liver 9000g supernatant fraction was prepared as described previously (6). Livers from a male human donor (age 65 (6)), male Sprague Dawley CD Charles River rats (Charles River Breeding Laboratories, Inc., St. Wilmington, MA) and NMRI-mice (Anticimex, Stockholm, Sweden) were used. Incubations were performed at a liver protein concentration of 3 mg/ml in the presence of MgCl_2 (10 mM), glucose-6-phosphate (40 mM), NADP^+ (1.8 mM), and glucose-6-phosphate dehydrogenase (1.2 units/ml). The incubation media were gently shaken at 37°C

under either carbogen, ^{16}O - or ^{18}O -atmosphere. The technique for ^{18}O -incubations will be described elsewhere².

(22R)-[22,23,24,25- ^3H]Budesonide (5 μM) and [^3H]butyraldehyde (5 μM) were separately incubated with liver preparations up to 30 min, and samples (1 ml) taken and immediately frozen on dry ice. To each sample was added 1 ml of 2,4-DNPH solution (see above). After thawing and mixing, the samples were allowed to stand at 4°C for 1 h. One millilitre of 75% (v/v) methanol in water was added, and after 30 min the samples were centrifuged at 1300g for 15 min. The supernatant fractions were filtered through Millipore HV disposable filters (pore size 0.45 μm) and samples of 500 μl injected on the HPLC column (see below).

For the identification of butyric acid, a mixture of the (22R)-budesonide (45 μg , 0.1 μmol) and ($^2\text{H}_8$)budesonide (45 μg) was incubated with liver 9000g supernatant fraction (10 ml). After incubation the liver homogenate was deproteinized by addition of an equal volume of 2 M HCl and centrifuged at 2200g for 20 min. About 7 ml of the supernatant fraction was saturated with NaCl and extracted with 5 ml of diethyl ether. The sample was centrifuged at 1500g for 5 min and the ether extract dried with Na_2SO_4 . The ether was evaporated to dryness under a stream of nitrogen at room temperature and the residue silylated with 20 μl of TMSI. About 3 μl was used for analysis by gas chromatography mass spectrometry (GC-MS).

In one experiment, a mixture of (22R)-budesonide (225 μg , 0.5

μmol), (22R)-($^2\text{H}_8$)budesonide (225 μg) and (22R)-[1,2- ^3H]budesonide (0.1 μg , 10 μCi) was incubated for 30 min with mouse liver 9000g supernatant fraction (50 ml) either in the absence or presence of esterase inhibitors (PMSF, 1 mM and BPNPP, 5 mM).

Prior to liquid chromatography mass spectrometry (LC-MS) analysis, samples were extracted on Sep-pak C_{18} cartridges (6). After incubation in the presence of esterase inhibitors, part of the extracted sample was separated by HPLC and radioactive peaks collected.

HPLC. The chromatographic equipment consisted of an automated injector (WISP model 710 B), a model 680 gradient controller, two M 6000 pumps, and a M 440 UV-detector ($\lambda = 254 \text{ nm}$) from Waters Associates (Milford, MA). UV-data were processed on a HP 3388 A integrator (Hewlett Packard, Avondale, PA). Radioactivity was detected on line with a Packard Trace 7140 (Packard, Downers Grove, Ill). Separation was performed on a 200 x 5 mm Nucleosil C_{18} 5 μm column (Macherey-Nagel, Düren, Germany). Spectrophotometric data were processed on a HP 3388 A integrator (Hewlett Packard, Avondale, PA). Butyric acid was detected at 210 nm and the 2,4-DNPH-derivative of butyraldehyde at 365 nm. In some experiments a refractive index detector was used to detect butanol. After 10 min of isocratic elution at 1 ml/min with 25% (v/v) methanol in 0.01 M phosphate buffer (pH 3.5), a gradient was run to 97.5% methanol in the same buffer during 50 min.

Mass spectrometric analysis. Mass spectra were obtained using a Finnigan (San José, California) 4500 instrument interfaced to a Finnigan INCOS data system.

Butyric acid was gas chromatographed and identified as its trimethylsilyl ester after reaction with TMSI according to ref. 13. The gas chromatographic column (180 cm x 2 mm I.D.) was packed with 3% OV-1 on Chromosorb, 60-80 mesh. It was operated at 40°C for 1 min after injection, and then the temperature was raised to 100°C at a rate of 5°C/min. The injector temperature was 100°C. Helium was used as carrier gas at a flow rate of 10 ml/min. Chemical ionization mass spectra were obtained using methane as reagent gas at an indicated ion source pressure of 0.4-0.5 torr. Data acquisition was started 2.5 min after injection and spectra were repetitively recorded between m/z 130 and 180.

A moving belt LC-MS interface (Finnigan MAT) with a polyimide belt was used for the on line LC-MS investigations. The methodology was identical to that used previously (6) and will be described in detail elsewhere².

Results and discussion

The metabolic acetal splitting of budesonide giving 16 α -hydroxyprednisolone requires NADPH. It is furthermore catalyzed by liver microsomal enzymes and inhibited by SKF 525A (5). Liver monooxygenases generally have these features, but an oxidative reaction cannot in a simple way explain the molecular events of the biotransformation. Budesonide, being chemically stable against hydrolysis (unpublished observations), may theoretically be hydrolyzed enzymatically to 16 α -hydroxyprednisolone and butyraldehyde.

To elucidate whether butyraldehyde was formed during the acetal cleavage, [22,23,24,25-³H]budesonide was incubated for 30 min at 37°C with liver 9000g supernatant fraction from rat, mouse and man in the presence of NADPH. In these experiments, using derivatization with 2,4-DNPH (12) and HPLC separation, no tritium labeled butyraldehyde could be detected. Instead a radioactive compound tentatively identified as butyric acid was formed. The formation of butyric acid was confirmed by incubating a mixture of budesonide and (²H₈)budesonide with liver preparations from rat, mouse and man. The trimethylsilyl derivatives of unlabeled and deuterium labeled butyric acid were identified by GC-MS in all investigated species. The possibility of rapid conversion of butyraldehyde to butyric acid was excluded, since only tritium labeled butanol could be detected after incubation of [³H]butyraldehyde with the same liver preparations. We therefore conclude that the formation of

butyric acid is an integral part of the metabolic $16\alpha,17\alpha$ -acetal side chain splitting of budesonide.

When the (22R)-epimer of $[1,2-^3\text{H}]$ budesonide was incubated with mouse liver 9000g supernatant fraction for 30 min, two metabolites could be detected (fig. 2A). These have previously been identified as 16α -hydroxyprednisolone and 6β -hydroxybudesonide (5). However, if esterase inhibitors were added prior to incubation, the formation of 16α -hydroxyprednisolone was inhibited. Instead a new peak appeared in the chromatogram (fig. 2B). When the compound corresponding to this peak, was isolated and incubated in buffer with porcine liver carboxylic ester hydrolase for 5 min at 37°C , the compound was completely hydrolyzed to 16α -hydroxyprednisolone. These results indicate that the $16\alpha,17\alpha$ -acetal splitting of budesonide proceeds via a previously unknown intermediary ester of 16α -hydroxyprednisolone, which in the presence of liver esterase activity instantaneously is hydrolyzed to 16α -hydroxyprednisolone and butyric acid.

To elucidate the structure and mode of formation of this intermediary ester, an incubation with mouse liver 9000g supernatant fraction was performed in the presence of esterase inhibitors, using the 22R-epimer of budesonide and $(^2\text{H}_8)$ budesonide. A sample from this incubation was analyzed by LC-MS. The chemical ionization mass spectrum of the intermediary compound (fig. 3A) showed peaks at m/z 447 and 454, which represent the protonated molecular ions expected after an oxidation of budesonide and

($^2\text{H}_8$)budesonide in the $16\alpha,17\alpha$ -acetal group. The difference of 7 u between the MH^+ ions of the unlabeled and deuterium labeled material showed that one of the eight deuterium atoms of the acetal moiety was lost. When a similar incubation was performed under ^{18}O -atmosphere, ^{18}O was incorporated in the intermediary compound (fig. 3B). The ions at m/z 389 and 396, remaining after loss of the 17β side chain ($\text{MH}^+-\text{CHOCH}_2\text{OH}$), also contained ^{18}O (c.f. figs 3A and 3B). The ion at m/z 359, remaining after loss of butyric acid from the protonated molecular ion, did, however, not contain ^{18}O . In a similar incubation performed under ^{18}O atmosphere but in the presence of esterase activity, ^{18}O was not incorporated in 16α -hydroxyprednisolone. No ester intermediate was detected in this experiment. Taken together, these findings show that ^{18}O must have been incorporated in the acetal group, resulting in the formation of the intermediary butyrate ester. The ions at m/z 429 and 436 in Fig. 3A are formed by loss of water from the MH^+ ions. The corresponding ions in Fig. 3B were also found at m/z 429 and 436, indicating loss of H_2^{18}O . A mechanism has been proposed for the loss of water from the molecular ion of ethyl acetate (14). Although one should be careful to extrapolate results from electron impact ionization to chemical ionization, the proposed mechanism offers an attractive explanation for the loss of H_2^{18}O from the ester intermediate (scheme 1). This mechanism is plausible only if the butyrate ester is attached in the 16-position and not in the 17-position, because it requires a CH_2 -group next to the alcohol oxygen of the ester group. The mechanism furthermore requires the ^{18}O -atom to be bound as acyl

oxygen at the 22-carbon. Comparison with authentic 16 α -butyryloxy-
prednisolone showed that the HPLC retention time was
identical to that of the ester intermediate and that their mass
spectra showed the same characteristic ions (fig 3C). The
results presented above strongly indicate that the ester
intermediate is 16 α -butyryloxy- α -prednisolone.

We have in this study presented data concerning the metabolic
16 α ,17 α -acetal splitting of budesonide, which are in accordance
with the mechanism presented in fig. 4. Atmospheric oxygen will
be incorporated at the 22-carbon of budesonide by a microsomal
monooxygenase, i.e. cytochrome P-450. The resulting hypotheti-
cal product, 16 α -hydroxy- α -prednisolone-16 α ,17 α -hemiortho-
butyrate, is expected to be chemically labile and will rearrange to the
energetically more favourable 16 α -butyryloxy- α -prednisolone
isomer. Similar labile hemioorthoester intermediates are
suggested to be formed in the acyl migration of glucocorticoid
17- and 21-esters (15,16). In the presence of liver esterase
activity, 16 α -butyryloxy- α -prednisolone is instantaneously
hydrolyzed to 16 α -hydroxy- α -prednisolone and butyric acid. The
high capacity of liver esterases may explain why the
intermediary ester previously has not been detected in liver
homogenates (5) or plasma¹ after budesonide administration. The
same mechanism as presented in fig. 4, has been suggested in
the cytochrome P-450-catalyzed oxidation of methylene-
-dioxyphenyl compounds (1,3-benzodioxoles) (17). In that study,
however, only the end products were identified.

The currently used symmetric 16 α ,17 α -acetonide glucocorticoids have a metabolically stable dioxolane group (5,18,19). The dioxolane 22-carbon of these compounds provides no site for hydroxylation, probably due to the lack of a hydrogen atom at this carbon. We suggest here, that the dioxolane group of a new topical glucocorticoid, budesonide, is submitted to extensive biotransformation by a mechanism, novel for 16,17-substituted glucocorticoids. The acetal splitting of budesonide increases the overall rate of inactivation and hereby reduces the risk for systemic side effects.

Acknowledgments

We wish to express our sincere gratitude to Mr. L.-I. Wickström, AB Draco, for skillful assistance with the experimental synthetic work and to Mr. J. Paulson, AB Draco, who performed the liquid chromatographic mass spectrometric analysis.

Footnotes:

Reprint requests to: Staffan Edsbäcker, M.S., Pharmacokinetic laboratory, AB Draco, Box 34, S-221 01 Lund, Sweden.

¹S. Edsbäcker and Å. Ryrfeldt, Systemic elimination of budesonide in man; metabolic aspects. In manuscript.

²C. Lindberg, J. Paulson and S. Edsbäcker, in manuscript.

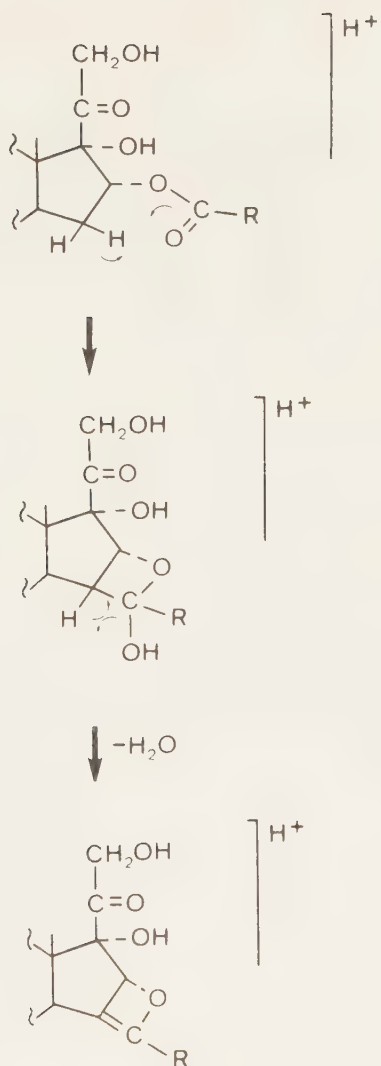
References

1. A.Heijer, G.Hesser, P.Holm and L.Salde: Comparison between two non-halogenated glucocorticoid ointments in psoriasis. *J.Int.Med.Res.* 9, 239-247 (1981).
2. S.P.Clissold and R.C.Heel: Budesonide - A preliminary review of its pharmacodynamic properties and therapeutic efficacy in asthma and rhinitis. *Drugs* 28, 485-518 (1984).
3. A.Thalén and R.Brattsand: Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with high local activity. *Arzneim.Forsch.Drug Res.* 29, 1687-1690 (1979).
4. R.Brattsand, A.Thalén, K.Roempke, L.Källström and E.Gruvstad: Influence of 16 α ,17 α -acetal substitution and steroid nucleus fluorination on the topical to systemic activity of glucocorticoids. *J.Steroid Biochem.* 16, 779-786 (1982).
5. S.Edsbäcker, S.Jönsson, C.Lindberg, Å.Ryrfeldt and A.Thalén: Metabolic pathways of the topical glucocorticoid budesonide in man. *Drug Metab.Disp.* 11, 590-596 (1983).
6. S.Edsbäcker, P.Andersson, C.Lindberg, J.Paulson, Å.Ryrfeldt and A.Thalén, Liver metabolism of budesonide in rat, mouse and man; comparative aspects. Submitted to *Drug Met.Disp.*

7. E.Dahlberg, A.Thalén, R.Brattsand, J.Å.Gustafsson, U.Johansson, K.Roempke and T.Saartok: Correlation between chemical structure, receptor binding and biological activity of some novel, highly active 16 α ,17 α -acetal substituted glucocorticoids. *Mol.Pharmacol.* 25, 70-78 (1984).
8. Å.Ryrfeldt, P.Andersson, S.Edsbäcker, M.Tönnesson, D.Davies and R.Pauwels: Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur.J.Resp.Dis.* 63, suppl. 122, 86-95 (1982).
9. S.Å.Johansson, K.E.Andersson, R.Brattsand, E.Gruvstad and P.Hedner: Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. *Eur. J. Clin. Pharmacol.* 22, 523-529 (1982).
10. A.Thalén: Epimers of budesonide and related corticosteroids. II. Structure elucidation by mass spectrometry. *Acta Pharm.Suec.* 19, 327-354 (1982).
11. J.W.Blunt and J.B.Stothers: ¹³C-N.m.r. spectra of steroids - A survey and commentary. *Org.Magn.Resonance* 9, 439-464 (1977).
12. K.Fung and D.Grosjean: Determination of nanogram amounts of carbonyls as 2,4-dinitrophenylhydrazones by high performance liquid chromatography. *Anal.Chem.* 53, 168-171 (1981).

- 13.O.A.Mamer and B.F.Gibbs: Simplified gas chromatography of trimethylsilyl esters of C₁ through C₅ fatty acids in serum and urine. Clin.Chem. 19, 1006-1009 (1973).
- 14.A.N.H.Yeo: Evidence for intra- and inter-alkyl hydrogen exchange in the mass spectra of ethyl acetate. Chem.Commun. 1154-1155 (1970).
- 15.H.Bundgaard and J.Hansen: Studies on the stability of corticosteroids VI. Kinetics of the rearrangement of betamethasone-17-valerate to the 21-valerate ester in aqueous solution. Int.J.Pharm. 7, 197-203 (1981).
- 16.T.Misaki, M.Yamada, R.Hirai, M.Komori, M.Washitake, Y.Ozawa, I.Koyama and H.Yoshizawa: Enzymatic hydrolysis of hydrocortisone diesters in skin. Yakuzaigaku 42, 92-98 (1982).
- 17.J.E.Casida, J.L.Engel, E.G.Essac, F.X.Kamienski and S.Kuwatsuka: Methylene-C¹⁴-dioxyphenyl compounds: metabolism in relation to their synergistic action. Science 153, 1130-1133 (1966).
- 18.K.J.Kripalani, A.I.Cohen, I.Weliky and E.C.Schreiber: Metabolism of triamcinolone acetonide-21-phosphate in dogs, monkeys, and rats. J.Pharm.Sci. 64, 1351-1359 (1975).

19.N.I.Chu, B.A.Amos, L.Tökes, M.L.Maddox, S.B.Matin, K.M.Hama, J.W.Paterson, P.J.Wagner, J.P. Bell and M.D.Chaplin: Disposition of flunisolide in the rat, mouse, dog, rhesus monkey and cynomolgus monkey. Drug Metab.Disp. 7, 81-89 (1979).



Scheme 1

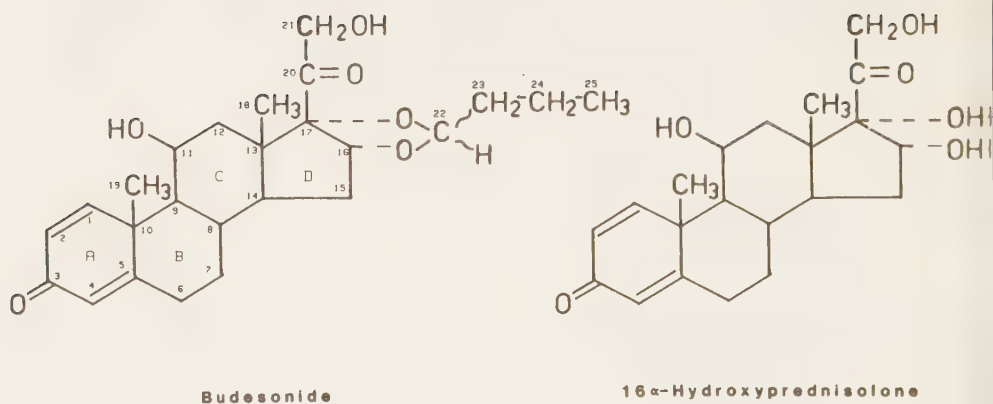


FIG. 1. Chemical structure of budesonide and its major metabolite, 16α-hydroxyprednisolone.

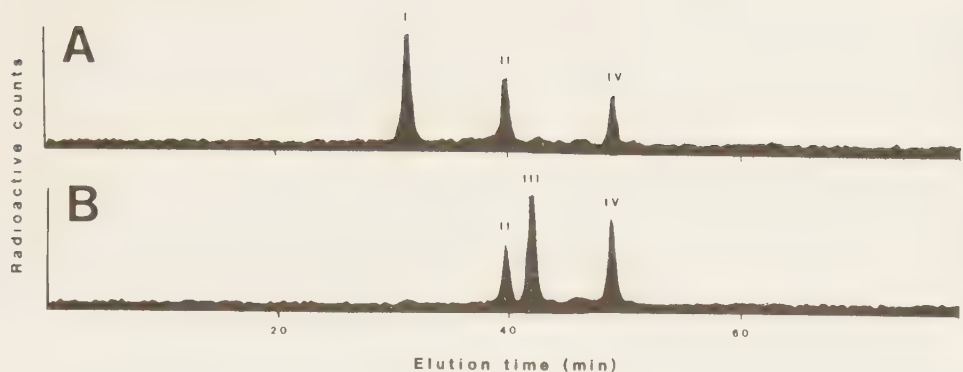


FIG. 2. Radiochromatograms obtained after incubation of (22R)-[1,2-³H]budesonide with mouse liver 9000g supernatant fraction in the absence (A) and presence (B) of esterase inhibitors.

The separated compounds correspond to 16 α -hydroxyprednisolone (I), 6 β -hydroxybudesonide (II), (22R)-budesonide (IV) and the intermediary compound (III). After extraction by Sep-pak C₁₈, separation was achieved on a Nucleosil C₁₈ column using a gradient of methanol in 0.01 M phosphate buffer as mobile phase at a flow rate of 1 ml/min.

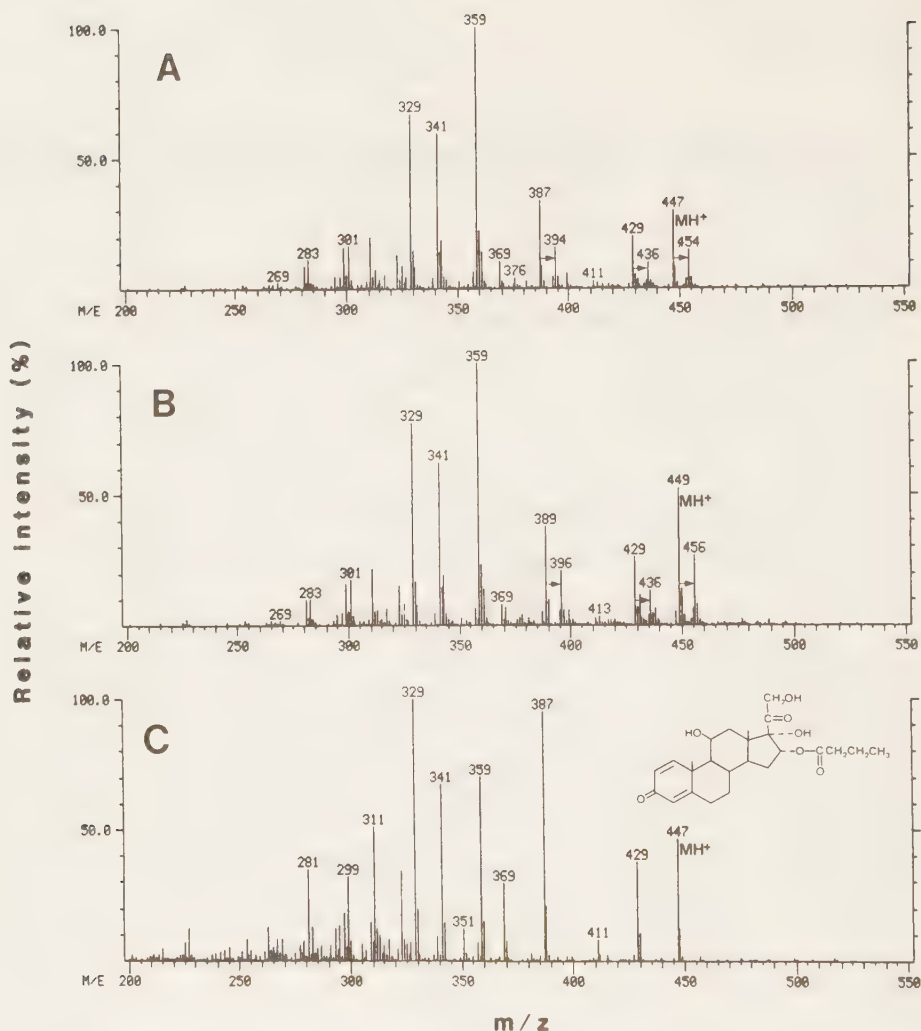


FIG. 3. Chemical ionization (CH_4) mass spectra of **A**; the intermediary ester obtained after incubation in an atmosphere containing ^{16}O , **B**; the intermediary ester obtained after incubation in an atmosphere containing ^{18}O and **C**; authentic 16 α -butyryloxyeprednisolone.

Arrows indicate deuterium containing ions. The incubations of the (22R)-epimer of budesonide and ($^2\text{H}_8$)budesonide were performed with mouse liver 9000g supernatant fraction in the presence of esterase inhibitors. After Sep-pak C_{18} extraction, LC-MS was run as described previously (6).

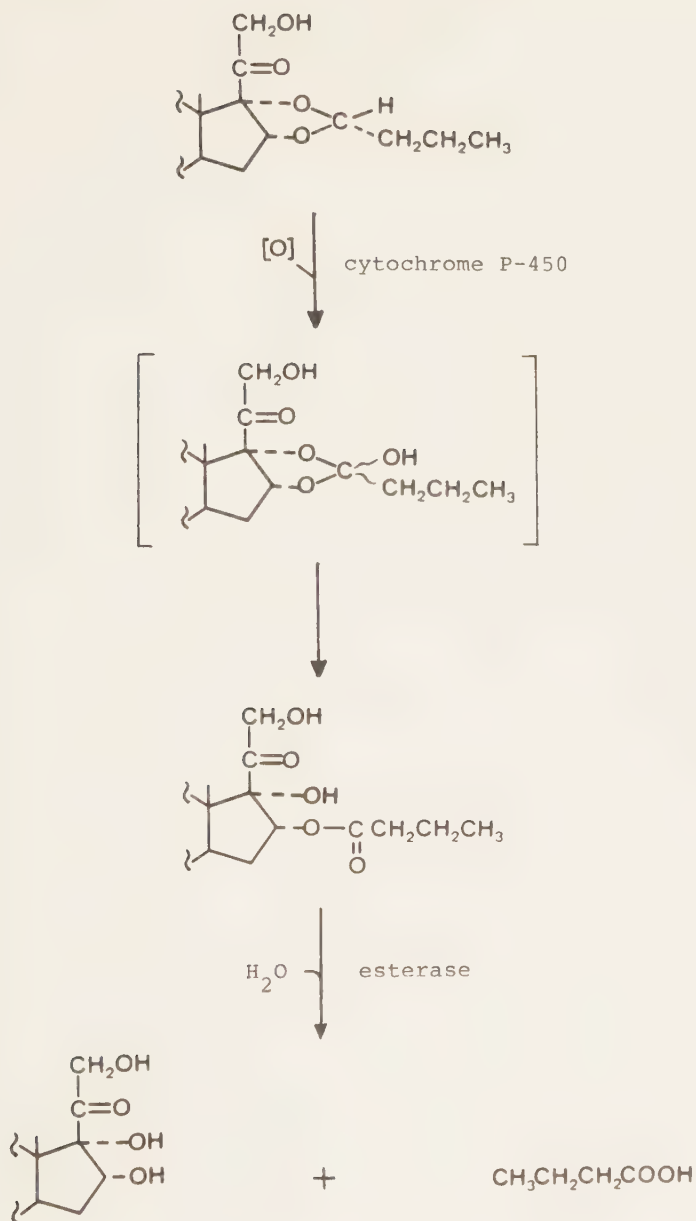


FIG.4. Suggested mechanism for the 16 α ,17 α -acetal splitting of (22R)-budesonide.

Structure in brackets denote a postulated hemiorthoester intermediate.





Nasal Bioavailability and Systemic Effects of the Glucocorticoid Budesonide in Man

S. Edsbäcker^{1,2}, K.-E. Andersson¹, and Å. Ryrfeldt^{1,2}

¹Department of Clinical Pharmacology, University Hospital, Lund and ²Research and Development Department, AB Draco, Lund, Sweden

Summary. Budesonide, a topically potent glucocorticoid, was administered to 4 healthy volunteers by i.v. infusion and by nasal instillation of 100 µg tritium-labelled drug. Plasma was analyzed by liquid chromatography plus scintillation counting of collected fractions. After i.v. administration the plasma clearance was 0.92 l/min and the apparent volume of distribution was 2.8 l/kg. After nasal administration, the time to reach the peak plasma level was approximately 30 min, and the systemic availability was 102%. Budesonide had marginal effects on plasma cortisol and white blood cell counts either after i.v. or nasal administration. Thus, nasally instilled budesonide in solution is rapidly and completely absorbed from the nasal mucosa. The systemic effects after this clinically recommended nasal dose were negligible.

Key words: budesonide, glucocorticoid; nasal administration, pharmacokinetics, bioavailability, systemic effects

Budesonide (Fig. 1) is a topically potent glucocorticoid, used clinically in the treatment of respiratory diseases, such as asthma [1] and rhinitis [2]. The drug is at least as effective as beclomethasone dipropionate [3] and flunisolide [4] in the local treatment of seasonal allergic rhinitis. In clinical trials, 400 µg budesonide daily was efficient in the treatment of hay fever, although 100 µg daily has been shown to give equally good protection against nasal allergen challenge [5]. In previous pharmacokinetic studies with ³H-budesonide in healthy volunteers, plasma clearance was estimated to be 1.4 l/min and the bioavailability after oral administration to be 10.4% [6]. No intact drug was detected in the urine. The data suggest that budesonide is a drug with a high hepatic clearance. The rapid liver biotransformation to metabolites with greatly reduced glucocorticoid activity

[7, 8], results in minimal adverse systemic effects of the drug.

Biotransformation of steroid hormones is known to occur in the lung [9, 10] and in the nose [11], both being important target organs for local treatment with glucocorticoids. Budesonide is not metabolized in human lung in vitro [10], but whether the nasal mucosa can metabolize the drug is not known.

In the present investigation, plasma kinetics and systemic availability were estimated after nasal and intravenous administration of ³H-budesonide to healthy male volunteers. In order to minimize gastrointestinal deposition, nasal instillation with a micropipette was preferred to the use of a spray or aerosol adapter. In addition, the effects of budesonide on plasma cortisol and white blood cell (WBC) counts were studied after both routes of administration.

Materials and Methods

Tritiated budesonide (³H-budesonide; (22RS)-16 α , 17 α -butylenedioxy-11 β ,21-dihydroxy-[1,2-³H]pregna-1,4-diene-3,20-dione) with a specific activity of 84.6 mCi/mg (3.13 GBq/mg) was obtained from the Radiochemical Centre, Amersham, UK. The compound was purified by the method of Andersson et al. [12]. A bulk solution was prepared by diluting labelled budesonide dissolved in ethanol with unlabelled compound to a specific activity of 0.5 mCi/mg. The infusion solution was made by diluting part of the bulk solution with saline (0.9% w/v NaCl) and ethanol to a final concentration of 10 µg/ml ³H-bu-

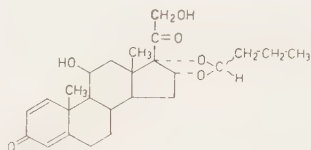


Fig. 1. Structural formula of budesonide

desonide and 10% (v/v) ethanol. The nasal solution was made by evaporating part of the bulk solution under a gentle stream of N_2 and dissolving the residue in a solution containing pH-regulating additives (pH = 5), preservatives and 10% (w/w) Tween 20 to give a final concentration of 1 mg/ml 3H -budesonide. Placebo solutions were prepared in a similar way but without the active drug. HPLC analysis of the infusion and nasal 3H -budesonide solutions showed a radiochemical purity of >95%. Unlabelled budesonide was obtained from Dr. A. Thalén, AB Draco. Ethanol was of spectroscopic grade and water was millipore-filtered (Millipore Super-Q, 4 module system). Acetic acid (Merck) was of p.a. quality. All HPLC solvents were degassed before use.

Four healthy male volunteers, 22–46 years old, and weighing 63–95 kg participated in the study. They were informed about the nature of the study and gave their written consent to it. The study protocol was approved by the Ethics committee of the University of Lund. The subjects were healthy, as shown by routine medical examination including haematological and biochemical tests. All were non-smokers. No drugs or alcohol were consumed for 4 days before or during the day of drug administration. All meals during the days of 3H -budesonide administration were standardized.

The subjects were given placebo and 3H -budesonide via nasal instillation and intravenous infusion, in a cross-over fashion. On Days 1 and 15 each subject received placebo, and the following day (Days 2 and 16) each subject received 3H -budesonide. All doses were given within 30 min of 8 a.m. The subjects were free to move and work during the trial, except for 30 min prior to and 2 h after each administration, when they were recumbent.

Placebo or 100 μ g (230 nmol, 50 μ Ci) 3H -budesonide was administered either by infusion over 5 min of 10 ml sterile infusion solution (exact dose estimated by weighing the syringe before and after administration), or by instillation into each nostril with a calibrated micropipette of 50 μ l nasal solution. To assure uniform distribution on the nasal mucosa, the instillations were followed by head turning at one-minute intervals, 0–10 min after administration, and at 5-min intervals, from 10–30 min after administration. Blood samples (10 ml) were collected immediately before and 30 min, and 2, 4, and 8 h after placebo administration. 20–25 ml blood samples were taken immediately before and 5, 15, 30, and 45 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h after 3H -budesonide administration. Heparinized blood sampling tubes were used throughout the study. Plasma was quickly separated from cells and immediately frozen in dry ice (-60°C). Samples were stored at -20°C until analyzed.

3H -budesonide Assay

Three standard plasma samples containing 3H -budesonide in the range 0.2–7 nmol/l were prepared for each assay. Each plasma assay comprised pre-treatment and analysis of plasma samples from a single subject for one route of administration plus a blank and 3 standard samples. To 5 ml plasma was added 6 ml internal standard, containing 18 μ mol unlabelled budesonide in 0.01 M acetic acid/ethanol 9/10. The sample was mixed on a sample mixer and was centrifuged at 1000 g for 10 min. 10 ml supernatant was applied to a preconditioned (5 ml ethanol followed by 5 ml 0.01 M acetic acid) Sep-Pak C_{18} cartridge (Waters Associates, Milford, USA). The cartridge was washed with 5 ml 10% (v/v) ethanol in 0.01 M acetic acid and the sample eluted with 4 ml ethanol. The eluate was evaporated at 50°C under a gentle stream of nitrogen and the residue dissolved in 400 μ l 25/75 (v/v) ethanol/water. 200 μ l was injected on to the HPLC column. All steps were performed at ambient temperature. Aliquots were taken throughout the extraction for calculation of recovery. The ethanol eluate recovery of radioactivity after Sep-Pak extraction of standard plasma samples was $94.2 \pm 7.2\%$ ($\bar{x} \pm \text{SD}$, $n = 24$).

Analysis was performed by HPLC as described previously [13]. A 45 min solvent program was run on a Nucleosil C_{18} -column, using a linear gradient from 25 to 62.5% (v/v) ethanol in water followed by a 5-min column wash with ethanol. One minute fractions were collected for measurement of radioactivity.

Radioactivity was measured by the liquid scintillation technique in Optifluor (10 ml), using a PA 3255 (Packard Instruments, Downers grove, USA) liquid scintillation counter equipped with an external standard for determination of counting efficiency. Each scintillation vial was counted for 2×4 min giving a CV for each counting occasion of <20%. The detection limit, determined by the number of counts corresponding to twice the background radioactivity, was approximately 0.1 nmol/l.

Standardization was performed with the HPLC-analysis of each assay. The radioactivity corresponding to 3H -budesonide was divided by the area of the internal standard budesonide UV-peak. A standard curve was made after calculation of the radioactivity/UV-area ratio for each standard sample containing known amounts of 3H -budesonide. Inter-assay variability, expressed as the variation in the slope of the standard curve was 4.1% (CV, $n = 8$). The intercept variation was 0.02 nmol/l (SD, $n = 8$) and the correlation coefficients in all cases were >0.999.

Plasma Cortisol and White Blood Cell (WBC) Counts

Five plasma samples from each subject given 3H -budesonide were analyzed for cortisol and WBC (total

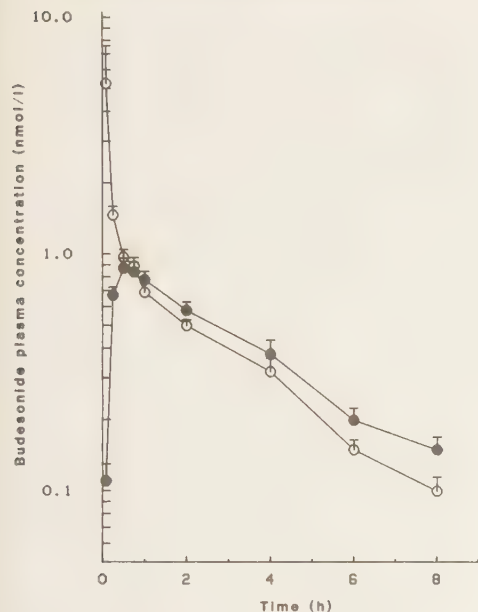


Fig. 2. Mean ($\pm$ SEM) plasma concentration of ^3H -budesonide after nasal ($\bullet$) and intravenous ($\circ$) administration of $100\ \mu\text{g}$ ($50\ \mu\text{Ci}$) ^3H -budesonide to 4 healthy subjects

and differential count) as previously described [13]. ^3H -budesonide and its metabolites may theoretically cross-react in the cortisol radioimmunoassay. However, the plasma concentration of total radioactivity expressed in ^3H -budesonide equivalents never exceeded 2.5% of the plasma cortisol concentration after placebo administration.

Pharmacokinetic and Statistical Calculations

The terminal slope (β) of the plasma concentration-time curve was determined by linear regression analysis. The area under the plasma concentration-time curve ($\text{AUC}_{0-8\text{ h}}$) was determined by the trapezoidal rule using the scheduled sampling times. The extrapolated area ($\text{AUC}_{8-\infty\text{ h}}$) was calculated as $C_{8\text{ h}}/\beta$. The plasma clearance (CL) was calculated as $\text{dose}_{\text{i.v.}}/\text{AUC}_{\text{i.v.}(0-\infty\text{ h})}$ and the distribution volume (V_β) as CL/β . Mean residence time (MRT) was calculated as $\int_0^\infty t \cdot C_p \cdot dt / \text{AUC}_{(0-\infty\text{ h})}$ and mean absorption time (MAT) as $\text{MRT}_{\text{nasal}} - \text{MRT}_{\text{i.v.}}$. The V_{ss} was calculated as $\text{dose}_{\text{i.v.}} \cdot \text{MRT}_{\text{i.v.}} / \text{AUC}_{\text{i.v.}(0-\infty\text{ h})}$. Systemic availability was calculated as $(\text{AUC}_{\text{nasal}(0-\infty\text{ h})} / \text{dose}_{\text{i.v.}}) / (\text{AUC}_{\text{i.v.}(0-\infty\text{ h})} / \text{dose}_{\text{nasal}})$.

Table 1. Pharmacokinetics of ^3H -budesonide in 4 healthy male subjects given $100\ \mu\text{g}$ ^3H -budesonide i.v. and by nasal instillation

	Subject				Mean $\pm$ SD
	I	II	III	IV	
Dose [nmol]					
i.v.	218.3	217.4	216.1	219.4	217.8 ± 1.4
nasal	198.2	206.4	206.4	198.2	202.3 ± 4.7
$\text{AUC}_{0-8\text{ h}}$ [nmol/l·h]					
i.v.	3.53	3.63	4.63	2.90	3.67 ± 0.72
nasal	3.86	3.38	3.45	2.36	3.26 ± 0.64
$\text{AUC}_{8-\infty\text{ h}}$ [nmol/l·h]					
i.v.	0.31	0.59	0.24	0.33	0.37 ± 0.15
nasal	0.47	0.68	0.59	0.39	0.53 ± 0.13
CL [l/min] ^a	0.95	0.86	0.74	1.3	0.92 ± 0.17
V_{ss} [l] ^a	165	200	90.3	207	166 ± 53.4
V_β [l] ^a	189	220	137	266	203 ± 54.1
[l/kg]	2.96	3.33	2.18	2.80	2.82 ± 0.48
$t_{1/2}$ [h]					
i.v.	2.30	2.96	2.14	2.71	2.53 ± 0.37
nasal	2.52	3.14	3.24	2.53	2.86 ± 0.38
MRT_{v} [h] ^a	2.93	3.92	2.08	3.09	3.01 ± 0.75
MAT [h]	0.71	0.65	2.05	0.77	1.04 ± 0.67
Nasal t_{max} [min]	30	30	45	15	
Availability [%]	124	101	86.8	94.4	102 ± 16.1

^a estimated from intravenous data

The differences between placebo and drug treatment values for WBC and plasma cortisol were tested using the paired two-tailed *t*-test. Other statistical calculations employed the unpaired two-tailed *t*-test.

Results

The plasma concentrations of ^3H -budesonide after nasal and intravenous administration are given in Fig. 2. The mean 12, 24 and 48 hour plasma concentrations of the drug were below the detection limit (0.1 nmol/l). Peak plasma values of ^3H -budesonide were reached 30 min (range 15–45 min) after nasal administration, indicating rapid absorption of the drug through the nasal mucosa. Pharmacokinetic data based on calculation of the plasma levels of ^3H -budesonide are given in Table 1. CL, V_β and $t_{1/2}$ i.v. were estimated to be 0.92 l/min, 2.8 l/kg and 2.5 h respectively. The systemic availability after nasal administration of ^3H -budesonide was about 100% (range 87–124%). The effect of budesonide on white blood cell counts and plasma cortisol is shown in Table 2. A slight depression of total WBC, eosinophils and plasma cortisol was found after both routes of administration, but the effect was marginal and only occasionally was it significant.

Table 2. Changes in white blood cell count and plasma cortisol after intravenous and nasal administration of placebo and 100 µg ³H-budesonide to 4 healthy subjects. Mean ± SD

Time after adm. [h]	WBC [10 ⁹ /l]		Neutrophils [%]		Eosinophils [%]		Lymphocytes [%]		Plasma cortisol [µg %]	
	Placebo	³ H-BUD	Placebo	³ H-BUD	Placebo	³ H-BUD	Placebo	³ H-BUD	Placebo	³ H-BUD
Intravenous administration										
0	4.0 ± 0.3	5.6 ± 0.4	54.4 ± 6.2	55.8 ± 2.9	1.7 ± 1.1	1.8 ± 1.4	37.7 ± 6.8	36.8 ± 4.6	21.1 ± 4.8	19.3 ± 6.2
0.5	5.8 ± 0.8	5.4 ± 0.7 ^a	58.9 ± 3.5	60.4 ± 3.6	2.0 ± 1.1	2.0 ± 1.6	33.8 ± 4.0	32.3 ± 3.8	12.1 ± 3.6	12.5 ± 4.4
2.0	6.2 ± 0.8	6.2 ± 0.5	57.0 ± 5.8	59.3 ± 5.8	2.5 ± 1.8	1.7 ± 1.3	35.2 ± 6.4	34.3 ± 4.4	13.2 ± 3.2	7.7 ± 2.3 ^a
4.0	6.6 ± 0.8	5.9 ± 0.6 ^b	56.1 ± 3.7	59.0 ± 2.7	1.8 ± 1.3	1.1 ± 0.7	36.8 ± 4.7	34.9 ± 4.2	12.3 ± 2.8	8.7 ± 3.9
8.0	7.1 ± 0.5	6.3 ± 1.2 ^b	54.1 ± 1.6	62.7 ± 4.1 ^a	1.5 ± 1.0	1.2 ± 1.0 ^a	39.5 ± 2.0	32.3 ± 4.8 ^a	10.4 ± 4.2	10.6 ± 4.2
Nasal administration										
0	5.4 ± 0.2 ^b	5.5 ± 0.7	56.3 ± 8.0	58.9 ± 3.1	2.1 ± 1.4	2.2 ± 1.4	34.2 ± 7.6	31.1 ± 5.0	18.8 ± 7.8	18.6 ± 4.3
0.5	5.4 ± 0.7	5.5 ± 0.8	60.4 ± 5.0	61.0 ± 3.3	2.7 ± 1.8	2.0 ± 0.7	31.1 ± 4.8	29.4 ± 4.5	13.9 ± 3.5	12.6 ± 3.7
2.0	5.8 ± 0.6	5.8 ± 0.8	60.7 ± 4.9	56.4 ± 4.2	2.0 ± 1.5	1.5 ± 1.6	30.0 ± 2.7	34.3 ± 6.0	11.8 ± 3.7	7.1 ± 2.2
4.0	6.4 ± 0.3	6.1 ± 1.1	56.4 ± 4.8	54.1 ± 4.0	2.0 ± 1.1	1.3 ± 1.3	34.5 ± 5.6	37.9 ± 6.5	13.3 ± 4.4	10.0 ± 5.7
8.0	7.0 ± 0.6	6.6 ± 0.9	57.8 ± 6.1	56.6 ± 3.3	1.5 ± 1.3	1.6 ± 1.4	33.5 ± 6.9	34.0 ± 5.1	11.8 ± 4.8	9.8 ± 2.2

^a *p* < 0.05^b Mean ± SD of three subjects

Discussion

Few data have been published about the availability of steroids after nasal administration. The nasal mucosa, being extensively vascularized, should normally exert a limited barrier for the absorption of lipophilic compounds such as steroids. However, the rat nasal mucosa contains several steroid-metabolizing enzymes [14], and progesterone has been reported to be metabolized after *in vitro* incubation with slices of nasal mucosa from rat and mouse [11]. The pharmacokinetics of flunisolide have been studied after nasal administration [15]. Dissolved drug was administered via a metered dose pump, and the dose was estimated to be 270 nmol (117 µg) by weighing each container before and after dosing. The peak plasma concentration of unchanged flunisolide was 1.4 ± 0.4 nmol/l (mean ± SD, *n* = 12) and was reached 15 min after administration. This should be compared with the peak budesonide concentration of 0.9 ± 0.2 mol/l found 30 min after deposition of 200 nmol into the nasal mucosa. The bioavailability of flunisolide in the subjects was calculated to be 49%, possibly indicating that some of the drug underwent nasal metabolism, or that only part of the administered drug was absorbed through the nasal mucosa. In the present study, the bioavailability after nasal instillation of budesonide was 102 ± 16% (mean ± SD), indicating either no or negligible metabolism of the drug during absorption through the nasal mucosa.

Ryrfeldt et al. [6] have estimated the bioavailability of budesonide after bronchial inhalation. Tritiated drug was administered in micronized form by means of the Inhaler device [6]. After correction for the amounts deposited in the oral cavity and the aerosol adapter, the bioavailability was 73 ± 42% (mean ± SD, *n* = 3). Since budesonide is not metabolized in human lung *in vitro* [10], the remaining frac-

tion was most probably swallowed and eliminated via first-pass metabolism in the liver. When budesonide was administered via bronchial inhalation, the peak plasma level was reached within 30 min after administration, which is close to the *t*_{max} found in the present study.

Budesonide is intended for the local treatment of asthma and rhinitis. From this and previous studies of the kinetics and metabolism of the drug, it may be concluded that budesonide is readily absorbed from the respiratory tract with no or negligible local biotransformation.

Budesonide may be classified as a high hepatic clearance drug, since the renal clearance is negligible and the hepatic extraction ratio is almost 90% [16]. Elimination of parenterally administered drug is in this case limited by the hepatic perfusion rate rather than the metabolic activity of the liver [17]. Variation in liver blood flow will markedly influence plasma clearance and may contribute to intraindividual variation in pharmacokinetics, including bioavailability. In the present study, plasma clearance after *i.v.* administration (100 µg) was 0.9 ± 0.2 l/min. In a previous study, in which 500 µg ³H-budesonide was given to 6 healthy volunteers [6], plasma clearance was 1.4 ± 0.5 l/min. The difference in CL between studies was not statistically significant (*p* = 0.124). The volume of distribution (*V*_β) in the present study (2.8 ± 0.5 l/kg) was, however, significantly (*p* < 0.008) lower than in the previous intravenous study (4.3 ± 0.7 l/kg). Plasma half-lives were similar in both studies - 2.5 ± 0.4 h here as compared to 2.8 ± 1.1 h in the previous study. The reason for the inter-study variation in *V*_β and possibly also in CL are not known, but may include pharmacogenetic and methodological discrepancies. In this context, the small number of subjects should also be stressed. Non-linear plasma protein binding [18–24] and saturation of glucocorticoid receptors [25] have been suggested as

explanations of the dose-dependent kinetics noted for prednisolone. Whether such effects contribute to the possible dose-dependency of budesonide kinetics in the dose-range investigated (100–500 µg) remains at present unsettled.

The treatment of non-infectious rhinitis as well as asthma with topical glucocorticoids has been shown to be effective and to be associated with minimal or no systemic side effects [26, 27]. In general, only a small fraction of an inhaled drug is absorbed at the target site, and probably the major fraction is swallowed and absorbed via the gastrointestinal tract [28]. Extensive first pass metabolism to inactive metabolites will then strongly restrict untoward systemic effects. The systemically available portion of the drug dose will still produce some systemic glucocorticoid effects if the dose is sufficiently high [13, 15, 29]. The effects on plasma cortisol and WBC after nasal administration of 100 µg budesonide, a clinically recommended dose [30], were marginal.

In conclusion, budesonide dissolved in 10% Tween 20 and applied to the nasal mucosa by instillation is rapidly and extensively absorbed into the systemic circulation. It undergoes negligible or no local biotransformation and has negligible systemic effects. The influence of the formulation on the clinical efficacy of budesonide remains to be investigated.

Acknowledgements. We thank Mss. A.-B. Eriksson, M. Nilsson and S. Ragnarsson for their excellent assistance and E. Owman for typing the manuscript.

References

1. Ellul-Micallef R, Johansson SÅ (1980) Budesonide: A new corticosteroid in bronchial asthma. *Eur J Respir Dis* 61: 167–173
2. Pipkorn U, Rundcrantz H, Lindqvist N (1980) Budesonide – a new nasal steroid. *Rhinology* 18: 171–175
3. Pipkorn U, Rundcrantz H (1982) Budesonide and beclomethasone dipropionate in hay fever – a single-blind comparison. *Eur J Respir Dis* 63 [Suppl 122]: 221–230
4. Pipkorn U, Geterud Å (1984) A comparative trial testing budesonide and flunisolide nasal sprays in patients with seasonal allergic rhinitis. *Ann Allergy* 52: 183–186
5. Pipkorn U (1982) Budesonide and nasal allergen challenge testing in man. *Allergy* 37: 129–134
6. Ryrfeldt Å, Andersson P, Edsbäcker S, Tönnesson M, Davies D, Pauwels R (1982) Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis* 63 [Suppl 122]: 86–95
7. Edsbäcker S, Jönsson S, Lindberg C, Ryrfeldt Å, Thälén A (1983) Metabolic pathways of the topical glucocorticoid budesonide in man. *Drug Metab Dispos* 11 (6): 590–596
8. Dahlberg E, Thälén A, Brattsand R, Gustafsson JÅ, Johansson U, Roempeke K, Saartok T (1984) Correlation between chemical structure, receptor binding and biological activity of some novel highly active 16 α , 17 α -acetal substituted glucocorticoids. *Mol Pharmacol* 25: 70–78
9. Hartiala J (1976) Steroid metabolism in adult lung. *Agents Actions* 6/4: 522–526
10. Andersson P, Ryrfeldt Å (1984) Biotransformation of the topical glucocorticoids budesonide and beclomethasone 17 α , 21-dipropionate in human liver and lung homogenate. *J Pharm Pharmacol* 36: 763–765
11. Brittebo EB (1982) Metabolism of progesterone by the nasal mucosa in mice and rats. *Acta Pharmacol Toxicol* 51: 441–445
12. Andersson P, Edsbäcker S, Ryrfeldt Å, von Bahr C (1982) In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J Steroid Biochem* 16: 787–795
13. Ryrfeldt Å, Edsbäcker S, Pauwels R (1984) Kinetics of the epimeric glucocorticoid budesonide. *Clin Pharmacol Ther* 35: 525–530
14. Brittebo EB, Rafter JJ (1984) Steroid metabolism by rat nasal mucosa; studies on progesterone and testosterone. *J Steroid Biochem* 20: 1147–1151
15. Chaplin MD, Cooper WC, Segre EJ, Dren J, Jones RE, Nerenberg C (1980) Correlation of flunisolide plasma levels to eosinopenic response in humans. *J Allergy Clin Immunol* 65 [6]: 445–453
16. Wilkinson GR, Shand DG (1975) A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 18 [4]: 377–390
17. Nies AS, Shand DG, Wilkinson GR (1976) Altered hepatic blood flow and drug disposition. *Clin Pharmacokinet* 1: 135–155
18. Bergrem H, Grøttum P, Rugstad HE (1983) Pharmacokinetics and protein binding of prednisolone after oral and intravenous administration. *Eur J Clin Pharmacol* 24: 415–419
19. Legler UF, Frey FJ, Benetz LZ (1982) Prednisolone clearance at steady state in man. *J Clin Endocrinol Metab* 55: 762–767
20. Loo JCK, McGilveray IJ, Jordan N, Moffat J, Brien R (1978) Dose dependent pharmacokinetics of prednisone and prednisolone in man. *J Pharm Pharmacol* 30: 736
21. McAllister WAC, Windfield CR, Collins JV (1981) Pharmacokinetics of prednisolone in normal and asthmatic subjects in relation to dose. *Eur J Clin Pharmacol* 20: 141–145
22. Meffin PJ, Brooks PM, Sallustio BC (1984) Alterations in prednisolone disposition as a result of time of administration, gender and dose. *Br J Clin Pharmacol* 17: 395–404
23. Pickup ME, Lowe JR, Leatham PA, Rhind VM, Wright V, Downie WW (1977) Dose dependent pharmacokinetics of prednisolone. *Eur J Clin Pharmacol* 12: 213–219
24. Rose JQ, Yurchak AM, Jusko WJ (1981) Dose dependent pharmacokinetics of prednisone and prednisolone in man. *J Pharmacokinet Biopharm* 9: 389–416
25. Tanner A, Bochner F, Caffin J, Halliday J, Powell L (1979) Dose dependent prednisolone kinetics. *Clin Pharmacol Ther* 25: 571–578
26. Mygind N (1982) Topical steroid treatment for allergic rhinitis and allied conditions. *Clin Otolaryngol* 7: 343–352
27. Johansson SÅ, Andersson KE, Brattsand R, Gruvstad E, Hedner P (1982) Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. *Eur J Clin Pharmacol* 22: 523–529
28. Newman SP, Pavia D, Morén F, Sheahan NF, Clarke SW (1981) Deposition of pressurized aerosols in the human respiratory tract. *Thorax* 36: 52–55
29. Harris DM (1975) Some properties of beclomethasone dipropionate and related steroids in man. *Postgrad Med J* 51 [Suppl 4]: 20–25
30. Balle VÅ, Pedersen V, Engby B (1982) The treatment of perennial rhinitis with a new non-halogenated, topical, aerosol packed steroid, budesonide. *Acta Otolaryngol* 94: 169–173

Received: November 3, 1984

accepted: March 13, 1985

Staffan Edsbäcker, M.S.

AB Draco

Research and Development Department

P.O. Box 34

S-221 00 Lund, Sweden



V



Kinetics of the epimeric glucocorticoid budesonide

Budesonide is a potent nonhalogenated glucocorticoid consisting of a 1/1 mixture of the two epimers 22R and 22S. The kinetics of these epimers were studied in six healthy male subjects after intravenous administration of 500 μg ^3H -budesonide. Estimation of the epimer concentrations in plasma was made possible by development of an HPLC method for simultaneous separation and quantification. The plasma $t_{1/2}$, distribution volume (V_{β}), and plasma clearance for epimer 22R were ($\bar{X} \pm \text{SD}$) 2.66 ± 0.57 hr, 425 ± 100 l, and 117 ± 40 l/hr. The corresponding values for epimer 22S were 2.71 ± 0.69 hr, 245 ± 38 l, and 67 ± 19 l/hr. Differences in V_{β} and plasma clearance between the two were significant. The larger V_{β} noted for epimer 22R may be a result of higher tissue affinity. The high plasma clearance for both epimers should largely reflect their high rate of liver biotransformation.

Ake Ryrfeldt, Ph.D., Staffan Edsbäcker, M.S., and Romain Pauwels, M.D.

Lund, Sweden, and Ghent, Belgium

AB Draco, Research and Development Laboratories, and Department of Clinical Pharmacology, University Hospital of Lund, Lund, and Department of Respiratory Diseases, Academic Hospital, Ghent

Budesonide is a potent nonhalogenated corticosteroid that contains an asymmetric $16\alpha,17\alpha$ -acetal group. It has high topical anti-inflammatory potency but low systemic activity.^{4, 5, 16} Budesonide was shown to be clinically effective in local treatment of respiratory and skin disorders.^{8, 10, 12, 18} The separation between high topical activity and low systemic effect is probably due to rapid inactivation by liver biotransformation of the drug to low activity metabolites.^{1, 4, 7} The basic kinetics of tritiated budesonide (^3H -bud) given intravenously, orally, or by inhalation have been reported in man.¹¹ Plasma $t_{1/2}$ was calculated to be somewhat more than 2 hr, which, compared to other potent glucocorticoids, can be considered as a short $t_{1/2}$.¹⁵ Systemic availability after oral doses was found

to be about 10%, indicating extensive first-pass metabolism.

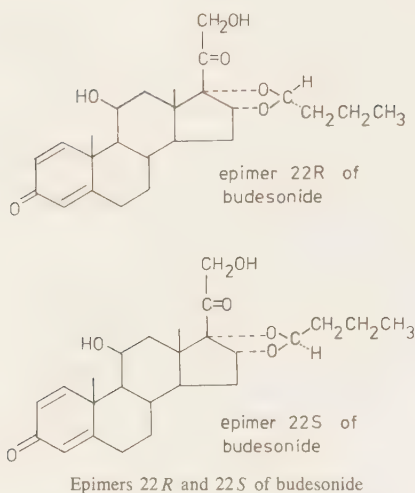
Budesonide is a 1/1 mixture of the epimers 22R and 22S, both of which seem to have about the same qualitative pharmacologic effects, but epimer 22R is two to three times as potent as epimer 22S.⁵ In vitro experiments with liver supernatant (9000g) from mouse, rat, and man have shown that budesonide is rapidly biotransformed.¹ Epimer 22R was biotransformed about twice as rapidly as 22S in human liver. To elucidate the kinetics of each epimer in vivo, ^3H -bud was injected intravenously in man. This was made possible by the development of an HPLC method that could separate the two epimers.¹⁷ The effects of budesonide on plasma cortisol concentrations and white blood cell counts were also studied.

Methods

Tritiated budesonide (^3H -bud; (22R,S)- $16\alpha,17\alpha$ -butyridenedioxy-11 β ,21-dihydroxy-[1,2-

Received for publication Aug. 13, 1983; accepted Oct. 12, 1983.

Reprint requests to: Dr. Åke Ryrfeldt, AB Draco, Research and Development Laboratories, Box 1707, S-221 01 Lund, Sweden.



^3H]pregna-1,4-diene-3,20-dione) was obtained from Amersham. The infusion solution was prepared by diluting labeled budesonide dissolved in ethanol with unlabeled compound (obtained from Dr. Arne Thalén) and adding saline solution (0.9% w/v NaCl) to obtain appropriate specific activity and concentration. According to HPLC the radiochemical purity was 94.3%, specific activity was 8.0 MBq/mg, concentration was 25 $\mu\text{g}/\text{ml}$, and the epimeric ratio 22R/22S was 53/47. The concentration of ethanol in the infusion solution was 10%. Ethanol and dichloromethane were of spectroscopic grade, and water was filtrated by a Millipore Super-Q. All HPLC solvents were degassed before use.

Six adult men 24 to 25 yr old weighing 65 to 81 kg participated in the study. The subjects were healthy as shown by routine physical examinations and laboratory tests (hematologic and biochemical analyses). All were moderate consumers of alcohol. No alcohol was taken 2 wk before and during the study. The subjects did not use any drugs. One subject (No. 5) was a smoker (about 20 cigarettes a day), but the others were nonsmokers.

On day 1, 20 ml of sterile placebo solution containing 10% ethanol was infused during 10 min at 8 A.M. Blood samples were drawn at 0 and 30 min and 2, 4, and 8 hr after starting the

infusion. At 8 A.M. the following day 20 ml of the sterile ^3H -bud solution was infused during 10 min. The dose was 500 μg (100 μCi). Blood samples were drawn at 0, 10, 15, 30, and 45 min and 1, 2, 4, 6, and 8 hr after starting drug infusion. Urine was collected 0 to 24 hr after dosing. Plasma and urine samples were stored frozen (-20°) until assayed.

All plasma samples were measured for total radioactivity ($2 \times 400 \mu\text{l}$ aliquots). Quantification of ^3H -bud was according to the following modification of the procedure of Ryrfeldt et al.¹⁴: Four milliliters of plasma was added to 10 ml of an internal standard solution (11 $\mu\text{g}/\text{ml}$ unlabeled budesonide in a 0.2% ethanolic water solution). Dichloromethane (30 ml) was added to the solution, followed by 30 min of gentle shaking on an Edmund Buhler sample shaker. The mixture was then centrifuged at 2500 rpm for 20 min. Aliquots ($2 \times 1 \text{ ml}$) from the aqueous layer were measured for radioactivity. Twenty-five milliliters of the organic phase was evaporated at 40° under a stream of nitrogen, and residue dissolved in 200 μl of the mobile phase was used in HPLC analysis. Aliquots ($2 \times 25 \mu\text{l}$) were measured for radioactivity and 100 μl was injected on the HPLC column. In this way 10 plasma samples from each subject and three standards (i.e., plasma with ^3H -bud added in the range 1.5 to 22 nmol/l) were extracted in 1 day and analyzed the next. All steps were performed at ambient temperature. Recovery in the organic phase as tested by extraction of standard plasma samples was $106.8\% \pm 6.2\%$ ($\bar{X} \pm \text{SD}$; $n = 18$). Total recovery of radioactivity in the extraction of plasma samples was $102.1\% \pm 4.1\%$ ($n = 49$).

Urine samples collected at 0 to 24 hr from each subject were used in the determination of renal excretion of unchanged ^3H -bud. Ten milliliters of each urine sample was applied to a $9 \times 90 \text{ mm}$ column (model K9, Pharmacia Fine Chemicals) packed with 100 to 200 μm XAD-2 (Serva Feinbiochemica). The column was connected to a model 10200 peristaltic pump (LKB) calibrated to a flow of 1.45 ml water/min. The column was washed with 45 ml water and the sample was eluted with 30 ml methanol. Recovery of radioactivity in the methanol phase was $98.4\% \pm 1.0\%$ ($n = 6$). The eluate was evaporated on a Rotavapor (Büchi) and the resi-

due was dissolved in 200 μ l of a 70/30 (v/v) water/ethanol solution containing 200 μ g/ml unlabeled budesonide. Fifty milliliters was injected on the HPLC column.

The liquid chromatograph was a model 660 solvent programmer with two M6000 A pumps and a M440 UV-detector ($\lambda = 254$ nm) from Waters. A model CV-6-UHPa-N60 injector (Valco) equipped with a 250- μ l loop and a model B100 fraction collector (Gilson) were also used. Analysis was performed on a 5 $\times$ 50 mm Corasil C₁₈ precolumn (Waters) connected to a 5 $\times$ 200 mm analytical column that was slurry packed with Nucleosil C₁₈, 5- μ m particles (Macherey-Nagel). A water/ethanol mixture (55/45, v/v) was used as mobile phase at a flow rate of 1 ml/min, giving a pressure of 3500 psi. This chromatographic system gave baseline separation between the budesonide epimers.¹⁷ The chromatographic recovery of injected radioactivity was 92.4% $\pm$ 9.8% (n = 44). In the urine analysis a 45-min solvent program was performed with a linear gradient from 25% to 60% (v/v) ethanol in water. Recovery of radioactivity through the HPLC procedure was 95.7% $\pm$ 2.0% (n = 6).

Radioactivity measurements were performed in Instagel with a TRI-CARB 460 CD liquid scintillation counter. Counting efficiency was estimated by the external standard procedure. To assure satisfactory counting precision, each scintillation vial was counted for 2 $\times$ 10 min, maximum 2 $\times$ 1600 counts. This method gave a percent SD range for each counting occasion of 2.5% to 10%, and also an acceptable correlation between the two counting occasions for the low-radioactivity scintillation vials.

Five plasma samples from each subject given ³H-bud were analyzed for cortisol and counted for white blood cells (WBC, total and differential). Controls were obtained by administration of placebo solution to the same subject the day before drug injection. Plasma concentrations of cortisol were determined by radioimmunoassay (Rianen cortisol I-125) with an LKB Wallac 80 000 gamma counter. The ³H-bud and its metabolites may theoretically cross-react in the cortisol assay, but the plasma concentration of total radioactivity never exceeded 10% of the cortisol plasma concentration after placebo.

Standardization with each analysis was by a

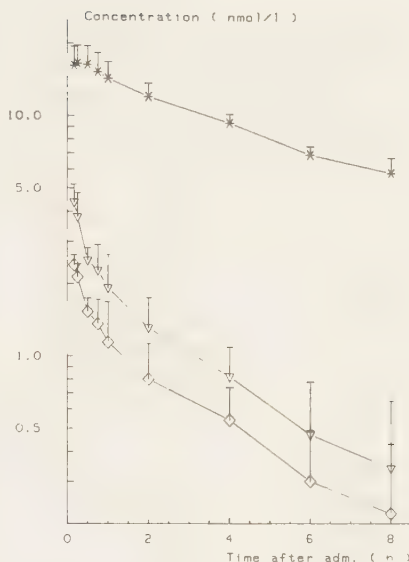


Fig. 1. Plasma concentrations ($\bar{X} \pm$ SD) of epimers 22S (∇) and 22R ($\circ$) of total radioactivity (*). The ³H-bud (500 μ g, 1.16 μ mol) was given to six healthy men as a 10-min intravenous infusion at time 0.

modified method of Ryrfeldt et al.¹⁴ The ratio of each ³H-bud epimer radioactivity peak to each internal standard epimer ultraviolet peak was calculated. In this way each epimer could be quantified separately. The standard curves used in the quantification of the ³H-bud epimers had correlation coefficients higher than 0.9999. The terminal slope (β) of the plasma concentration (C)-time curve was determined by linear regression analysis. The area under the plasma concentration-time curve ($AUC_{0-8 \text{ hr}}$) was determined by the trapezoidal rule. The remaining area ($AUC_{8-\infty \text{ hr}}$) was calculated as $C_{8 \text{ hr}}/\beta$. The plasma clearance (Cl) was calculated as dose/ AUC_{tot} and the distribution volume (V_{β}) as Cl/β . The paired two-sided t test was used for statistical examination.

Results

Mean plasma concentration profiles of total radioactivity and of each epimer of budesonide are shown in Fig. 1. The concentration of epi-

Table I. Kinetic parameters of the epimers 22R and 22S of budesonide

Subject No.	Weight (kg)	AUC (nmol/l × hr)		<i>t</i> _{1/2} (hr)		Cl (l/hr)		<i>V</i> _β (l)	
		22R	22S	22R	22S	22R	22S	22R	22S
1	81	5.56	9.24	2.43	3.00	106.6	61.7	373.5	267.0
2	70	4.80	7.33	2.33	2.36	123.6	77.8	416.0	264.5
3	70	11.56	19.10	3.78	3.98	51.3	29.8	279.6	171.3
4	73	4.63	7.59	2.69	2.48	128.1	75.1	497.9	268.3
5	68	3.40	6.94	2.25	2.24	174.6	82.1	567.5	265.7
6	65	5.08	7.77	2.46	2.20	116.7	73.4	415.1	233.5
$\bar{X}$	71	5.84	9.66	2.66	2.71	116.8	66.7	424.9	245.1
SD	±5	±2.89	±4.69	±0.57	±0.69	±39.8	±19.3	±99.6	±38.0

Table II. Effects on WBC counts and plasma cortisol concentrations

Time (hr)	Total WBCs (×1000/mm ³)		Eosinophils (mm ⁻³)		Neutrophils (%)		Lymphocytes (%)		Cortisol (μg/ml)	
	Placebo	³ H-bud	Placebo	³ H-bud	Placebo	³ H-bud	Placebo	³ H-bud	Placebo	³ H-bud
0	6.9	6.5	185	246	41	42	43	41	211	205
	±1.5	±1.0	±128	±177	±7	±10	±8	±12	±51	±32
0.5	6.7	6.1	181	152	40	44	41	39	177	151
	±1.1	±1.2*	±156	±76	±8	±7	±7	±7	±43	±41
2	6.9	7.0	130	128	40	52	46	30	104	83
	±1.1	±1.4	±59	±143	±6	±5	±4	±9†	±19	±25
4	7.3	7.0	141	81	38	57	48	31	103	49
	±1.1	±1.1	±53	±84	±4	±11†	±5	±9*	±35	±22*
8	7.5	8.4	111	77	55	46	31	38	75	43
	±1.5	±1.7	±27	±49	±5	±8	±8	±9	±24	±24*

Values are $\bar{X} \pm \text{SD}$.**P* < 0.05; †*P* < 0.01.

mer 22S was about 1.5 times that of epimer 22R at any specified sampling time. Accordingly, the AUC of epimer 22S was larger (*P* < 0.01) than that of epimer 22R (Table I). The two epimers had the same plasma *t*_{1/2}s, and both *V*_β and Cl of epimer 22R were larger (*P* < 0.01) than those of epimer 22S.

No or only traces of the unchanged epimers of budesonide could be detected in any of the six urine samples analyzed. The effects on WBC counts and plasma cortisol concentrations are listed in Table II. Plasma cortisol concentrations were slightly depressed by budesonide. A slight and transient decrease in total WBCs and lymphocytes was found as well as a slight increase in neutrophils. There were no statistically significant effects on plasma eosinophils.

Discussion

In our investigation the interest was focused on the fact that budesonide is a 1/1 mixture of the epimers 22R and 22S. Since epimer 22R had been found to be biotransformed in human liver (in vitro) about twice as rapidly as epimer 22S,¹ it was speculated that there might be a difference in kinetic profiles between the two epimers. In our study in healthy men it was found that the plasma *t*_{1/2}s of the two epimers were very close, but the *V*_β, and thus Cl of epimer 22R was almost twice as large as that of epimer 22S. These differences were significant. The larger *V*_β for epimer 22R may be explained by greater tissue affinity because of its greater hydrophobic properties. The solubility in water for epimer 22R is 8 μg/ml and that for epimer

22S is 23 $\mu\text{g/ml}$. Studies in isolated perfused lung model have shown that there is greater lung uptake (approximately 1.4 times) of epimer 22R than of epimer 22S from perfusion medium by rats and guinea pigs.*

Differences in binding to plasma proteins of the two epimers may also account for the observed differences in V_β . The protein binding of the 1/1 epimeric mixture of budesonide is 90%,¹³ but binding of the separate epimers has not been studied.

The major fraction (about two thirds) of ³H-bud excreted by man is eliminated by the kidneys.¹³ We found that no or only trace amounts were excreted in the urine (0 to 24 hr) as unchanged drug; thus urinary clearance of budesonide seems to approach or even exceed liver blood flow (about 90 l/hr). This may be due to extrahepatic clearance (metabolism) of budesonide, especially of epimer 22R. Another perhaps more probable explanation is that the extrapolated $\text{AUC}_{8-\infty}$ is underestimated, which would lead to overestimation in the calculation of Cl. Rate-limiting redistribution from tissues to plasma of a fraction of the tissue-distributed drug could have this effect on the plasma concentration-time curve. This property may be more accentuated for epimer 22R. Despite the possibility that there may be some overestimation of Cl of the epimers, it is likely that liver blood flow will be the limiting factor in the rate of plasma decline of budesonide epimers. The difference in biotransformation rates between the epimers found in vitro may therefore not be reflected in vivo when expressed as plasma $t_{1/2}$ s. Thus the kinetic profile of budesonide is very similar to that of the so-called high hepatic clearance drugs such as propranolol,^{2, 9} lidocaine,³ and alprenolol.⁹ Another glucocorticoid, flunisolide, is also reported to have a high hepatic clearance.⁶

In one subject (No. 3) there was a somewhat aberrant kinetic profile. The plasma $t_{1/2}$ of each epimer was longer and Cl and V_β were lower than in the five other subjects. Theoretic explanations include reduced liver blood flow and

lower capacity to metabolize the drug. Subject 5 was a smoker (about 20 cigarettes a day). He had the highest Cl for both epimers, but these values were very close to those of the other subjects. Cigarette smoking is not thought to affect elimination rates of glucocorticosteroids.¹¹

Effects on plasma cortisol and WBC counts were also studied. A slight depression of plasma cortisol levels was noted. The effects on WBC counts were those expected of a glucocorticoid, e.g., increase in neutrophils and decrease in lymphocytes, but these effects were small and transient. No significant interindividual variations on the effects on plasma cortisol and WBC counts were found.

Our investigation shows that there is a difference between the kinetic profiles of budesonide epimers in man. Both epimers can, however, be classified as high hepatic clearance compounds. Budesonide is a glucocorticoid effective in the local treatment of respiratory disorders such as bronchial asthma. Extensive liver biotransformation to low activity metabolites is an advantage in reducing systemic side effects.

References

1. Andersson P, Edsbäcker S, Ryrfeldt Å, von Bahr C: In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J Steroid Biochem* **16**:787-796, 1982.
2. von Bahr C, Hermansson J, Lind M: Oxidation of (*R*)- and (*S*)-propranolol in human and dog liver microsomes. Species differences in stereoselectivity. *J Pharmacol Exp Ther* **222**:458-462, 1982.
3. Benowitz N, Meister W: Clinical pharmacokinetics of lignocaine. *Clin Pharmacokinet* **3**: 177-201, 1978.
4. Brattsand R, Thalén A, Roempke K, Källstrand L, Gruvstad E: Development of new glucocorticosteroids with a very high ratio between topical and systemic activities. *Eur J Respir Dis* **63** (suppl 122):62-73, 1982.
5. Brattsand R, Thalén A, Roempke K, Källström L, Gruvstad E: Influence of steroid nucleus fluorination and type of 16 α ,17 α -acetal substitution on the topical to systemic glucocorticoid activity ratio of 16 α ,17 α -acetal corticoids. *J Steroid Biochem* **16**:779-786, 1982.
6. Chaplin M, Rooks W, Swenson E, Cooper W, Nerenberg C, Chu N: Flunisolide metabolism

*Ryrfeldt Å: Unpublished observations, 1983.

- and dynamics of a metabolite. *CLIN PHARMACOL THER* 27:402-413, 1980.
7. Edsbäcker S, Jönsson S, Lindberg C, Ryrfeldt Å, Thalén A: Metabolic pathways of the topical glucocorticoid budesonide in man. *Drug Metab Disp* 11:590-596, 1983.
 8. Heijer A, Hesser G, Holm P, Salde L: Comparison between two non-halogenated glucocorticoid ointments in psoriasis. *J Int Med Res* 9:239-247, 1981.
 9. Johansson G, Regårdh C-G: Clinical pharmacokinetics of β -adrenoceptor blocking drugs. *Clin Pharmacokinet* 1:233-263, 1976.
 10. Pipkorn U, Rundcrantz H, Lindqvist N: Budesonide—a new nasal steroid. *Rhinology* 18:171-175, 1980.
 11. Rose J, Yurchak A, Meikle M, Jusko W: Effect of smoking on prednisone, prednisolone and dexamethasone pharmacokinetics. *J Pharmacokinet Biopharm* 9:1-14, 1981.
 12. Rosenhall L, Lundqvist G, Ädelroth E, Glennow C: Comparison between inhaled and oral corticosteroids in patient with chronic asthma. *Eur J Respir Dis* 63(suppl 122):154-162, 1982.
 13. Ryrfeldt Å, Andersson P, Edsbäcker S, Tönneson M, Davies D, Pauwels R: Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis* 63(suppl 122):86-95, 1982.
 14. Ryrfeldt Å, Tönnesson M, Nilsson E, Wikby A: Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography. *J Steroid Biochem* 10:317-324, 1979.
 15. Swartz S, Dluhy R: Corticosteroids: Clinical pharmacology and therapeutic use. *Drugs* 16:238-255, 1978.
 16. Thalén A, Brattsand R: Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with high local activity. *Arzneim Forsch* 29:1687-1690, 1979.
 17. Wikby A, Nilsson L, Hållsås G: Separation of epimers of budesonide and related corticoids by reversed bonded-phase liquid chromatography. *J Chromatogr* 157:51-64, 1978.
 18. Willey R, Godden D, Carmichael J, Presten P, Frame M, Crompton G: Twice daily inhalation of a new corticosteroid, budesonide, in the treatment of chronic asthma. *Eur J Respir Dis* 63(suppl 122):138-142, 1982.

SERUM PROTEIN BINDING OF THE

BUDESONIDE EPIMERS IN MAN.

Staffan Edsbäcker and Kristina Geelmuyden

Pharmacokinetics Laboratory,

Research and Development Department,

AB Draco (Subsidiary of AB Astra)

Lund, Sweden.

ABSTRACT

The topical glucocorticoid budesonide is a 1:1 mixture of two epimers, 22R and 22S, having different disposition in man. Their protein binding in human serum was investigated by equilibrium dialysis, ultracentrifugation and ultrafiltration. Similar results were obtained by the three methods. Binding was constant within the investigated concentration range (0.5-250 nmol/L). Neither prednisolone nor the other budesonide epimer affected binding. Budesonide was displaced by cortisol only at very high cortisol concentrations. This indicates that budesonide is not bound to serum transcortin. Binding of (22R)-budesonide ($88.6 \pm 1.0\%$, mean $\pm$ SD, $n=12$) differed significantly ($p=0.0002$) from that of (22S)-budesonide ($90.7 \pm 1.5\%$). Similar difference was found with ultracentrifugation and ultrafiltration. The approximately 20% higher serum free fraction of (22R)-budesonide partly explains the larger volume of distribution of this epimer in man.

INTRODUCTION

Budesonide is a topically potent glucocorticosteroid, used clinically in the treatment of skin and respiratory diseases (Heijer et al 1981, Ellul-Micallef et al. 1980, Pipkorn et al. 1980). It is pharmacokinetically characterized by a large volume of distribution and high hepatic clearance (Ryrfeldt et al. 1982). The compound is not metabolized in human serum (Andersson and Ryrfeldt 1984). It distributes equally between plasma and erythrocytes (P Andersson, unpublished observations) and the protein binding has been estimated to approximately 90% (Ryrfeldt et al. 1982).

Budesonide is a 1:1 mixture of two epimers with 22R and 22S configuration (Fig.1). The disposition in man of the two budesonide epimers differ (Ryrfeldt et al. 1984):

	<u>Clearance (L/min)</u>	<u>V_{SS} (L)</u>
22R	1.95	408
22S	1.11	234

Effects of serum and tissue binding on disposition have been recognized for many drugs (Jusko and Gretch 1976). The protein binding in serum or plasma of the isolated epimers has previously not been investigated. The purpose of the present study was to find out whether different serum protein binding may explain the different pharmacokinetic profiles of the two budesonide epimers.

MATERIAL AND METHODS

Chemicals

[³H]budesonide ((22RS)-16 α ,17 α -butylidenedioxy-11 β ,21-dihydroxy [1,2-³H]pregna-1,4-diene-3,20-dione) with a specific activity of 3.13 GBq/mg was obtained from the Radiochemical Centre, Amersham, England. The (22R)- and (22S)-epimers were separated by HPLC (Edsbäcker et al. 1986). The radiochemical purity, determined by HPLC (see below), was 95.4% for (22R)-[³H]budesonide and 96.8% for (22S)-[³H]budesonide. Tritium-labelled compounds were diluted, when necessary, with unlabelled epimers, kindly supplied by Dr A Thalén, AB Draco. Stock ethanol solutions of the radioactive compounds were prepared and stored at -20°C. After evaporation, the compounds were redissolved in buffer or serum. Cortisol and prednisolone were obtained from Sigma Chemicals (St Louis, MO, USA). Water was filtered through a Millipore Super Q 4 module system. Other chemicals were of analytical grade.

Serum and buffers

Blood, obtained from healthy donors, were allowed to clot for one hour and then centrifuged for 15 minutes at 1000g. Serum was used fresh or within one month after storage at -20°C. Sera from some of the subjects were used after agitation with activated charcoal (Sigma Chemicals) for 1 h and subsequent filtration.

Phosphate buffers (0.028 M) of pH 6.7-7.7, made isotonic with NaCl, were used in the equilibrium dialysis experiments. Prior to use, and at the end of each experiment, buffer pH was measured by the Astrup micro technique (Radiometer, Copenhagen, Denmark).

Equilibrium Dialysis

Dialysis Plexi-glass cells as described by Ehrnebo et al (1971) were used, together with standard membranes Type A (Part no 933-0236-01) from Technicon Chemicals, Belgium. Buffer and drug-containing serum, 500 μ L, were introduced in each compartment which then were sealed with Teflon plugs. Tritium labelled compound (2.5 nmol/L, 40 μ Ci/nmol) was incubated for 4 h at 37°C, unless otherwise stated. Dialysis was performed by gentle axial rotation, keeping the membrane in a vertical position. The fraction of protein bound compound was calculated as $(C_s - C_b)/C_s$, where C_s and C_b are the concentration of radioactivity in serum and buffer respectively, at the end of incubation. The fraction of the compound which had been adsorbed to the Plexi-glass cell and/or the dialysis membrane, was calculated as $(C_{tot} - C_s - C_b)/C_{tot}$, where C_{tot} is the concentration of radioactivity in serum prior to dialysis. Each determination of binding was performed in duplicate.

Ultracentrifugation

Serum (1.5 mL) containing [3 H]-labelled compound was transferred into polycarbonate tubes and centrifuged for 8 hours at 37°C and 45.000 rpm (150.000 g) in a titanium angle rotor (No. 59592) using a MSE superspeed 65 Ultracentrifuge (MSE Ltd, London, England). The fraction of protein bound compound was calculated as $(C_{tot} - C_{free})/C_{tot}$, where C_{free} is the concentration of radioactivity in the clear layer at the top of the tubes after centrifugation. Each determination of binding was performed in duplicate.

Ultrafiltration

MPS 1 Starter Kit 4010 (Amicon Corp., MA., USA) with YMT-membranes was used as described by the manufacturer. Serum (500 μ L) containing [3 H]-labelled compound was centrifuged at 1000g for 10 min at ambient temperature. A small volume of ultrafiltrate was collected. The fraction of protein bound compound was then calculated as described for ultracentrifugation. Adsorption was checked by ultrafiltration of [3 H]-labelled compound dissolved in previously ultrafiltered blank serum.

HPLC

The equipment used for HPLC-analysis was principally the same as previously described (Andersson et al. 1983). Aliquots of the stock ethanol solutions of [^3H]-labelled budesonide epimers were diluted with water and injected on a 4.5 x 200 mm, 5 μm Nucleosil C_{18} column using 55/45 (v/v) water/ethanol as mobile phase. After budesonide elution, the column was washed for 5 min with pure ethanol. One minute fractions were collected during elution and washing, using a LKB 2211 Superrac (LKB produkter, Bromma, Sweden). After equilibrium dialysis of sera from two subjects, aliquots from the buffer and serum sides were diluted with phosphate buffer/ethanol and submitted to HPLC-analysis as above.

Cortisol analysis was performed in low to normal level serum, using the method of Van den Berg et al (1977) with slight modification.

Measurement of radioactivity

Serum and buffer samples and HPLC fractions were, after mixing with 10 mL Optiflour^R (Packard), analyzed for radioactivity with a liquid scintillation spectrometer (Packard Tri-Carb 300). Disintegrations were calculated using automatic external standardization.

Albumin analysis

The albumin concentration in human serum was determined by radial immunodiffusion (M-Partigen^R, Behringwerke).

Statistics

The pared two-sided t-test was used for statistical examinations.

RESULTS

Determination of serum protein binding of the budesonide epimers using equilibrium dialysis.

Experiments with pooled sera from three subjects showed that equilibrium was reached in about 2 hours. No change in binding was noted from 3 to 8 hours of incubation. There was furthermore no change in binding in sera which had been stored frozen (-20°C) as compared to fresh sera. The adsorption of radioactivity on the membrane and/or dialysis cell was similar for the two epimers (8.3 and 8.6%, mean in 12 subjects for epimers 22R and 22S). Serum binding of the budesonide C-22 epimers and serum albumin levels in 12 healthy subjects are given in Table 1. In all subjects, the binding of (22R)-budesonide was lower than that of (22S)-budesonide. Consequently, the serum free fraction of (22R)-budesonide (11.37%, mean in 12 subjects) was higher than that of (22S)-budesonide (9.26%). This difference ($2.11 \pm 1.03\%$, mean $\pm$ SD) was statistically significant ($p=0.00002$). There was no apparent correlation between binding and serum albumin levels.

Both buffer pH and incubation temperature affected serum protein binding. Binding of both epimers increased with increased buffer pH (Fig.2). At an initial buffer pH of 7.2, the pH at the end of the 4 h dialysis was 7.54 ± 0.03 (mean $\pm$ SD of both epimers in 12 subjects). When incubation temperature was lowered from 37°C to 24°C , the binding of both epimers increased by 0.7-1.1% (absolute values).

Radiochemical impurities influenced marginally the estimated serum binding. Dialyzed serum and buffer samples from two subjects (II and IV) were submitted to HPLC. The calculated binding after exclusion of radiochemical impurities increased slightly ($<0.4\%$), and similarly for both epimers.

Serum protein binding of (22R)- and (22S)-budesonide, determined at different concentrations of the ligand, is shown in Fig.3. There was no or minimal change in binding of the two epimers within the investigated concentration range.

Ultracentrifugation and ultrafiltration vs equilibrium dialysis

Serum protein binding of the budesonide epimers, determined by ultracentrifugation and ultrafiltration are given in Table 1. Adsorption of radioactivity to the Ultrafree^R device was similar for the two epimers (6-14%). The results indicate that the experimental technique does have an effect on the calculated binding of the budesonide C-22 epimers. The budesonide binding ((22R+22S)/2), estimated by ultracentrifugation ($89.41 \pm 0.40\%$, mean $\pm$ SD in 4 subjects), was similar to that obtained by equilibrium dialysis in the same subjects ($89.50 \pm 0.23\%$). The mean values, obtained by ultrafiltration ($91.64 \pm 1.10\%$, n=5), was higher than the corresponding values obtained by equilibrium dialysis ($89.78 \pm 1.49\%$). The difference in binding between the two epimers was more pronounced using ultracentrifugation ($3.53 \pm 0.76\%$) and ultrafiltration ($2.95 \pm 1.11\%$) as compared to equilibrium dialysis (see above).

Displacement experiments

Neither prednisolone ($1 \mu\text{g/mL}$, $3 \mu\text{mol/L}$), nor a 100-fold surplus of the other epimer, did influence serum protein binding of the budesonide epimers. Budesonide binding was not affected by the treatment of serum with activated charcoal, which reduced serum cortisol levels to $0.025 \mu\text{mol/L}$ (Fig.3). At very high serum cortisol levels, there was a decline in budesonide binding.

DISCUSSION

Cortisol and prednisolone bind to two serum proteins; albumin with low affinity and transcortin with high affinity (Ballard 1975). Transcortin has a binding capacity of approximately 0.7 μmol (250 ng) cortisol per L serum (Brien 1981). Serum or plasma protein binding of synthetic glucocorticoids, other than prednisolone, is confined primarily to albumin (Florini & Buyske 1961, Ballard 1975, Peets et al. 1969). Consequently, the binding is independent of concentration at plasma levels normally obtained after therapeutic doses. The constant binding of the budesonide epimers at different concentrations of the ligand, and at low ($<0.14 \mu\text{mol/L}$) to physiological ($0.14\text{--}0.8 \mu\text{mol/L}$) serum cortisol levels, indicates that budesonide is not bound to the high affinity transcortin. At the highest cortisol levels investigated (up to two-fold the normal concentration of serum albumin), saturation of serum albumin may be apparent. The decrease in budesonide binding then noted, suggest that budesonide is bound to the same albumin site(s) as cortisol.

The mean budesonide binding, determined by ultrafiltration, was higher than that obtained using ultracentrifugation or equilibrium dialysis. This can, at least partly, be explained by the lower temperature at which the ultrafiltration experiments were performed. Independent of experimental method, there was a statistically significant ($p < 0.005$) difference in protein binding between the two epimers.

Increased serum free fraction will increase volume of distribution (Jusko and Gretch 1976). For compounds with a high volume of distribution, a change in serum free fraction will change volume of distribution by about the same magnitude (cf. Gillette 1971 or Øie and Tozer 1979). The serum free fraction of (22R)-budesonide is more than 20% greater than that of the 22S-epimer. (22R)-Budesonide has a volume of distribution (V_{ss}), which is 74% larger than that of (22S)-budesonide (Ryrfeldt et al. 1984). Hence, the higher serum free fraction of (22R)-budesonide may partly explain the different distribution of the two epimers.

(22R)-budesonide is more extensively distributed into tissues than the 22S-epimer. Using isolated perfused rat or guinea-pig lungs, the tissue uptake of the 22R-epimer was higher (1.4 times) than that of (22S)-budesonide (Ryrfeldt et al., unpublished observations). A higher tissue uptake obviously contributes to the difference in distribution between the two epimers.

The biotransformation rate in human liver 9000g supernatant fraction is 2-3 times higher for (22R)- than for (22S)-budesonide (Andersson et al. 1983). Furthermore, the oral availability of (22R)-budesonide in man is less than half that of the 22S-epimer (Borgå et al., unpublished results). These data implies that the hepatic clearance of the two budesonide epimers differ.

In conclusion, the serum free fraction of (22R)-budesonide is significantly higher than that of (22S)-budesonide. This, together with a more extensive tissue distribution and higher hepatic clearance of the 22R-epimer, can explain the difference in disposition between the two epimers in man.

Acknowledgements. We are indepted to Dr A Thalén for supplying us with unlabelled budesonide epimers, to Mr H Lindberg for preparing and analyzing the low cortisol level sera and to Mrs E. Nikander for typing the manuscript.

REFERENCES

- Andersson P, Ryrfeldt Å (1984) Biotransformation of the topical glucocorticoids budesonide and beclomethasone 17 α ,21-dipropionate in human liver and lung homogenate. *J Pharm Pharmacol* 36: 763-765.
- Andersson P, Edsbäcker S, Ryrfeldt Å, von Bahr C (1983) In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. *J Steroid Biochem* 16: 787-795.
- Ballard PL (1975) Delivery and transport of glucocorticoids to target cells. *Monogr Endocrinol* 12: 25-48.
- Brien TG (1981) Human corticosteroid binding globulin. *Clin Endocrinol* 14: 193-212.
- Edsbäcker S, Andersson P, Lindberg C, Paulson J, Ryrfeldt Å, Thalén A (1986) Metabolism of budesonide in rat, mouse and man; comparative aspects.
- Ehrnebo M, Agurell B, Jalling B, Boréus LO (1971) Age differences in drug binding by plasma proteins; studies on human fetuses, neonates and adults. *Eur J Clin Pharmacol* 3: 189-193.
- Ellul-Micallef R, Johansson SÅ (1980) Budesonide: A new corticosteroid in bronchial asthma. *Eur J Respir Dis* 61: 167-173.
- Florini JR, Buyske DA (1961) Plasma protein binding of triamcinolone-³H and hydrocortisone-4-¹⁴C. *J Biol Chem* 236: 247-251.
- Gillette JR (1971) Factors affecting drug metabolism. *Ann N Y Acad Sci* 179: 43-66.

Heijer A, Hesser G, Holm P, Salde L (1981) Comparison between two non-halogenated glucocorticoid ointments in psoriasis. J Int Med Res 9: 239-247.

Jusko WJ, Gretch M (1976) Plasma and tissue protein binding of drugs in pharmacokinetics. Drug Met Rev 5: 43-140.

Pipkorn U, Rundcrantz H, Lindqvist N (1980) Budesonide - a new nasal steroid. Rhinology 18: 171-175.

Ryrfeldt Å, Andersson P, Edsbäcker S, Tönnesson M, Davies D, Pauwels R (1982) Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. Eur J Respir Dis 63 (suppl 122): 86-95.

Ryrfeldt Å, Edsbäcker S, Pauwels R (1984) Kinetics of the epimeric glucocorticoid budesonide. Clin Pharm Ther 35: 525-530.

Øie S, Tozer TN (1979) Effect of altered plasma protein binding on apparent volume of distribution. J Pharm Sci 68: 1203-1205.

Table 1. Serum protein binding of (22R)- and (22S)-budesonide.

Initial serum steroid concentration 2.5 nmol/L. Equilibrium dialysis were performed at 37°C for 4 hours, using isotonic phosphate buffer, initial pH 7.2, and sera from 12 healthy subjects. Values are mean from duplicate experiments. Ultracentrifugation, ultrafiltration and determination of serum albumin levels were performed as described in MATERIAL AND METHODS.

Subject	Sex	Albumin conc. (mg/mL)	Percent bound					
			Equilibrium dialysis		Ultracentrifugation		Ultrafiltration	
			22R	22S	22R	22S	22R	22S
I	F	33.9	88.4	90.8	88.2	91.1	90.0	92.4
II	M	30.1	88.2	90.7	86.8	91.3	89.2	93.7
III	M	57.3	88.7	89.7	87.3	90.9	n.d.	n.d.
IV	F	51.7	89.0	90.5	88.2	91.2	n.d.	n.d.
V	F	33.9	89.7	90.9	n.d.	n.d.	91.0	92.5
VI	M	43.0	90.6	93.1	n.d.	n.d.	91.9	94.9
VII	M	36.9	86.5	89.0	n.d.	n.d.	88.7	92.1
VIII	F	37.9	89.3	94.1	n.d.	n.d.	n.d.	n.d.
IX	F	52.7	88.1	90.3	n.d.	n.d.	n.d.	n.d.
X	F	44.0	88.2	89.5	n.d.	n.d.	n.d.	n.d.
XI	M	52.7	88.6	90.2	n.d.	n.d.	n.d.	n.d.
XII	M	48.3	88.3	90.0	n.d.	n.d.	n.d.	n.d.
Mean		43.5	88.6	90.7	87.6	91.1	90.2	93.1
±SD		9.0	1.0	1.5	0.7	0.2	1.3	1.2

n.d. = not determined

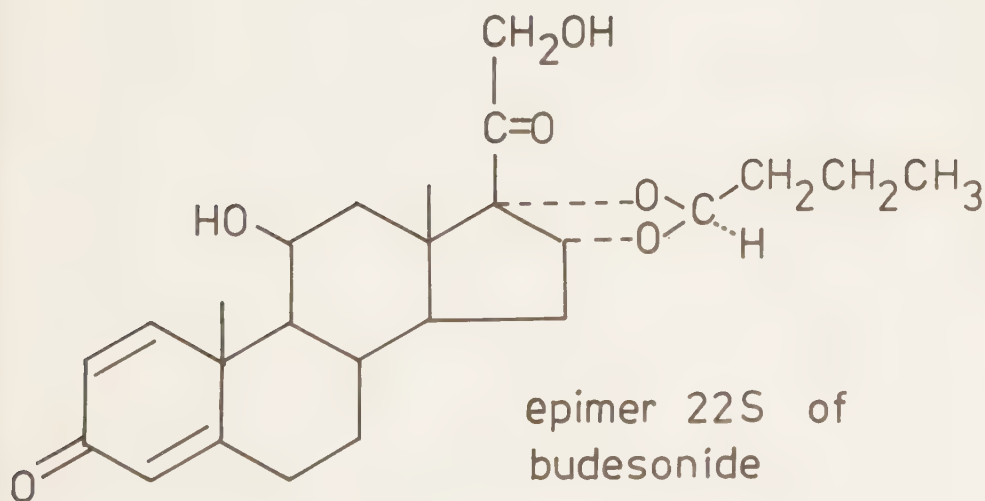
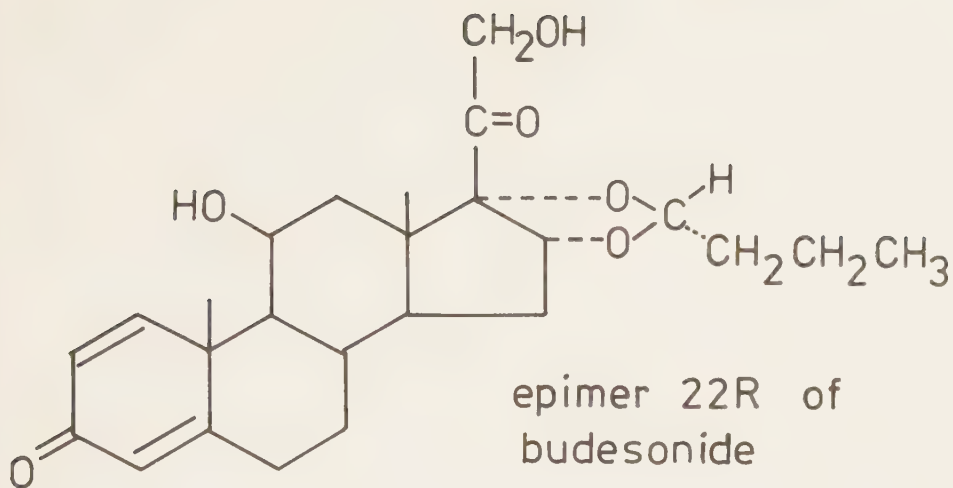


Fig.1. Structural formula of the budesonide epimers.

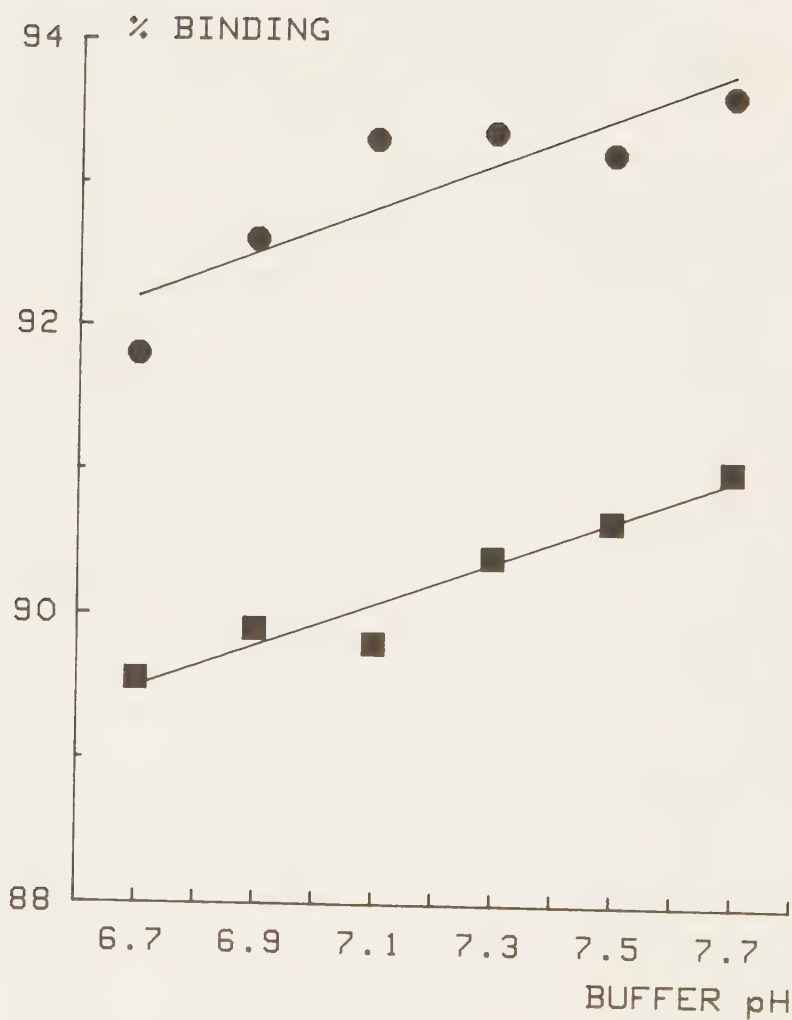


Fig.2. Serum protein binding of budesonide epimers 22R (■) and 22S (●) determined at different buffer pH. Initial serum budesonide concentration 4.5 nmol/L. Equilibrium dialysis was performed at 37°C for 4 h, using isotonic phosphate buffer pH 6.7-7.7. values are mean from duplicate experiments, using pooled sera from healthy subjects.

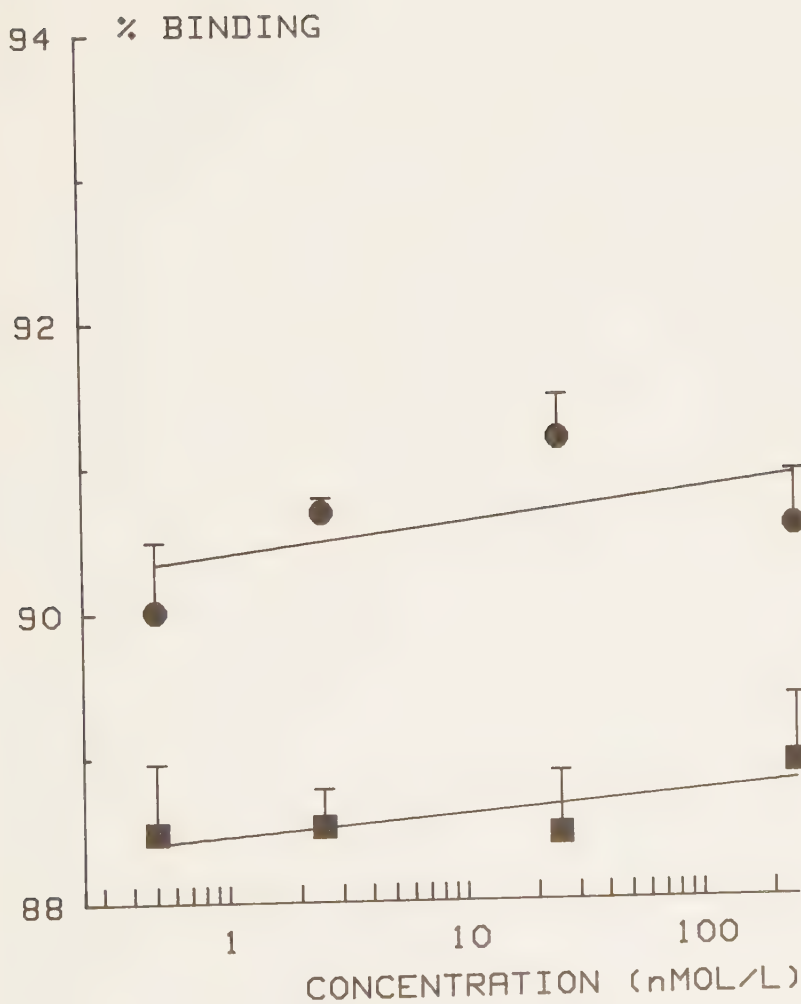


Fig.3. Serum protein binding of budesonide epimers 22R (■) and 22S (●) at different initial serum concentrations of the ligand. Equilibrium dialysis was performed at 37°C for 4 h, using isotonic phosphate buffer pH 7.2. Values are mean $\pm$ SEM from 3 subjects.

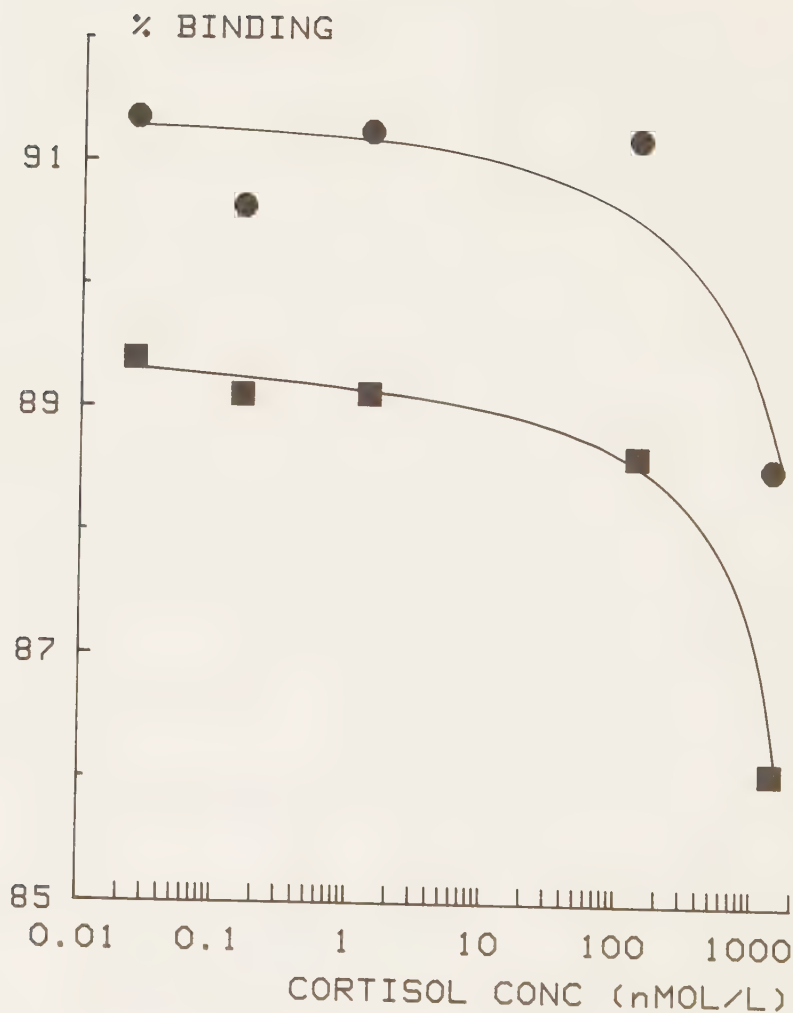


Fig.4. Serum protein binding of budesonide epimer 22R (■) and 22S (●) estimated at different cortisol concentrations. Initial serum budesonide concentration 2.5 nmol/L. Equilibrium dialysis was performed at 37°C for 4 h, using phosphate buffer pH 7.2. Values are mean from at least duplicate experiments, using pooled sera from healthy subjects.



